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EVALUATION OF METHODS FOR ANALYSIS OF HUMAN FAT, SKIN, NAILS, HAIR, BLOOD AND URINE

by

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16. ABSTRACT

This research program surveyed and evaluated the methods and procedures to identify and quantitate chemical constituents in human tissues and fluids including fat, skin, nails, hair, blood, and urine. These methods have been evaluated to determine their ease and rapidity, as well as cost, accuracy, and precision. During this evaluation, a second goal was to determine the feasibility of correlating a preferred method with a specific tissue/fluid and with easily identifiable chemical characteristics, such as octanol-water partition coefficient, water solubility, vapor pressure, etc.

Because of these goals, the search strategy, as well as the literature evaluation focussed on analytical methods. The literature search was restricted to lists of chemicals of current interest to the U.S. Environmental Protection Agency, to references cited in "Chemicals Identified in Human Biological Media, A Data Base," and the "Chemical Abstract Data Base."

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FOREWORD

The Exposure Assessment Group (EAG) of EPA's Office of Health and Environmental Assessment, Office of Research and Development, has three main functions: (1) to conduct exposure assessments; (2) to review assessments and related documents; (3) to develop guidelines for Agency exposure assessments. The activities performed under each of these functions are supported by and respond to the needs of the various EPA program offices. In relation to the third function, EAG sponsors projects which develop or refine data and procedures or techniques used in exposure assessments. This report is the product of one of those projects, and was done for the Office of Solid Waste and Emergency Response.

The current project was initiated to provide an evaluation of the analytical methods and procedures currently used to identify and quantify chemical constituents in selected human body tissues and fluids. Associated objectives were to determine if general correlations exist which relate preferred analytical method with chemical-physical properties of the analyte and with the tissue/fluid matrix being analyzed - and to determine which biological matrices serve as the best indicators of past or present exposures to the chemical constituents of concern. The results of this work provide basic guidance for the analysis of chemical compounds in specific body tissues and fluids, and suggest the meaningfulness of these analyses relative to the time frame of exposure and to body burden. The Exposure Assessment Group eventually hopes to provide more detailed guidance on biomonitoring.

James W. Falco
Director
Exposure Assessment Group

ABSTRACT

This research program surveyed and evaluated the methods and procedures used to identify and quantitate chemical constituents in human tissues and fluids including fat, skin, nails, hair, blood, and urine. These methods have been evaluated to determine their ease and rapidity, as well as cost, accuracy, and precision. During this evaluation, a second goal was to determine the feasibility of correlating a preferred method with a specific tissue/fluid and with easily identifiable chemical characteristics, such as octanol-water partition coefficient, water solubility, vapor pressure, etc.

Because of these goals, the search strategy, as well as the literature evaluation focussed on analytical methods. The literature search was restricted to lists of chemicals of current interest to the U.S. Environmental Protection Agency, to references cited in "Chemicals Identified in Human Biological Media, A Data Base," and the "Chemical Abstract Data Base."

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ABBREVIATIONS AND SYMBOLS

ASV	Anodic Stripping Voltammetry
AAS	Atomic Absorption Spectroscopy
CV	Coefficient of Variance
COL	Colorimetry
CV	Cyclic Voltammetry
cm^{-2}	Centimeter Square
dL	Deciliter
°C	Degrees Centrigrade
EC	Electrochemical Methods
ES	Emission Spectroscopy
eV	Electron Volts
EDXRF	Energy Dispersive X-Ray Fluorescence
γ	Gamma Rays
GC	Gas Chromatography
g	Gram
HPLC	High Performance Liquid Chromatography
HCL	Hollow Cathode Lamp
ICAP	Inductively Coupled Argon Plasma
IR	Infrared
KeV	Kilo Electron Volt
LOD	Limit of Detection
L	Liter
MS	Mass Spectrometry
mL	Milliliter
m/e	Mass-to-Charge Ratio
MeV	Mega Electron Volts
mg	Milligram
μL	Microliters
μmol/L	Micro mol per liter
μg	Microgram
mM	Millimolar
NAA	Neutron Activation Analysis
NBS	National Bureau of Standards
ng	Nanogram
nm	Nanometers
Φ	Neutron Flux
NMR	Nuclear Magnetic Resonance
PIXE	Proton Induced X-Ray Emission
ppb	Parts Per Billion
pg	Picogram
ppm	Parts Per Million
RLD	Reciprocal Linear Dispersion
RSD	Relative Standard Deviation
SRM	Standard Reference Material
sec	Second
TLC	Thin Layer Liquid Chromatography
t	Time
UV	Ultraviolet

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SECTION 1

INTRODUCTION

OVERVIEW

For a long time, air monitoring constituted the major means of assessing workers' exposure to chemicals in industry. But this monitoring considers only exposure by the pulmonary route and, even for chemicals that enter the organism mainly through inspired air, it does not indicate the true uptake by the exposed worker.

Biological monitoring, the routine analysis of human tissues or excreta for direct or indirect evidence of exposure to chemical substances, has also been used to learn more about early detection of health impairment due to industrial chemicals. The types of analyses include the following measurements:

- The concentration of the chemical in various biological media such as blood, urine, tissue and hair;
- The concentration of metabolites of the individual chemical in the same media; and
- Determination of nonadverse biological changes resulting from the reaction of the organism to exposure.

Hence, biological monitoring is used in the assessment of human exposure. A main goal of such monitoring is to ensure that the current or past levels of worker exposure are safe, so that such exposure does not involve an unacceptable health risk. Biological monitoring is being applied increasingly to the assessment of exposure in environmentally exposed nonworker populations.

A recent publication by Robert R. Lauwerys of the University of Louvain presents an excellent overview on the subject of biological monitoring guidelines (1). Another useful document was compiled by Randall C. Baselt (2).

The purpose of this research program was to survey and evaluate the methods and procedures used to identify and quantitate chemical constituents in human tissues and fluids including fat, skin, nails, hair, blood, and urine. These methods have been evaluated to determine their ease and rapidity, as well as cost, accuracy, and precision. During this evaluation, a second goal was to determine the feasibiltiy of correlating a preferred method with a specific tissue/fluid and with easily identifiable chemical characteristics, such as octanol-water partition coefficient, water solubility, vapor pressure, etc.

Because of these goals, the search strategy, as well as the literature evaluation, focussed on analytical methods. The literature search was restricted to lists of chemicals of current interest to the U.S. Environmental Protection Agency, to references cited in "Chemicals Identified in Human Biological Media, A Data Base" (3) up to 1982 and the "Chemical Abstracts Data Base." Table 1-1 shows the journals searched in the Chemicals Identified in Human Biological Media, A Data Base." The Chemical Abstract Data Base covers the complete chemical literature from 1967 to present, however, the search performed for this review was restricted to 1976 to 1983.

PROGRAM DESIGN

Selection of Target Chemicals

The list of target chemicals to be included in this review was compiled from a set of documents (4-7) received from the EPA Technical Project Monitor. The documents contained lists of compounds considered to be of interest to the EPA. Table 1-2 contains the total list of compounds searched for in this review and the compounds actually found in the computerized search.

The chemicals in Table 1-2 have been classified into a hierarchial scheme based on their physical and chemical properties. This scheme first separates inorganic from organic compounds. Organic chemicals are then separated according to the functional group substituents listed in Table 1-3. Functional groups which have the greatest effect on a compound's physical and chemical properties and/or analytical behavior are placed highest in the scheme. For example, carboxylic acids are ranked above acid esters. The acid moiety is a stronger

TABLE 1-1. JOURNALS SEARCHED FOR "CHEMICALS IDENTIFIED IN HUMAN BIOLOGICAL MEDIA, A DATA BASE"[a]

Acta Pharmacologica and Toxicologica
Air Pollution Control Association Journal
AMBIO
American Family Physician
American Industrial Hygiene Association Journal
American Journal of Clinical Nutrition
American Journal of Epidemiology
American Journal of Obstetrics and Gynecology
American Journal of Public Health
American Medical Association, Journal of the
Analytical Biochemistry
Analytical Chemistry
Analytical Toxicology, Journal of
Annals of Occupational Hygiene
Archives Internationales de Pharmacodynamine et de Therapie
Archives of Environmental Contamination and Toxicology
Archives of Environmental Health
Archives of Toxicology
Association of Official Analytical Chemists, Journal of the
Biochemical Journal
Biochemical Medicine
Biochemical Pharmacology
British Journal of Industrial Medicine
British Journal of Pharmacology
British Medical Journal
Bulletin of Environmental Contamination and Toxicology
Canadian Medical Association, Journal of the
Cancer Research
Chemosphere
Chronic Diseases, Journal of
Clinical Pharmacology, Journal of
Clinical Pharmacology and Therapeutics
Drug and Chemical Toxicology

(continued)

TABLE 1-1 (cont'd.)

Ecotoxicology and Environmental Safety
Environment
Environmental Health Perspectives
Environmental Pathology and Toxicology, Journal of
Environmental Pollution
Environmental Research
Environmental Science and Health, Journal of
Environmental Science and Technology
Epidemiology and Community Health, Journal of
Experientia
Food and Chemical News
Food and Cosmetics Toxicology
Forensic Sciences, Journal of
International Journal of Environmental Analytical Chemistry
International Journal of Environmental Studies
Japanese Journal of Pharmacology
Laboratory and Clinical Medicine, Journal of
Lancet
Medical Journal of Australia
Molecular Pharmacology
Mosquito News
Nature
Naunyn-Schmiedeberg's Archives of Pharmacology
New England Journal of Medicine
New York Academy of Sciences, Annals of the
Nutrition, Journal of
Nutrition Reports International
Obstetrics and Gynecology
Occupational Medicine, Journal of
Pediatrics
Pediatrics, Journal of
Pesticide and Toxic Chemical News

(continued)

TABLE 1-1 (cont'd.)

Pesticides Monitoring Journal
Pharmaceutical Sciences, Journal of
Pharmacological Reviews
Pharmacology and Experimental Therapeutics, Journal of
Pharmacy and Pharmacology, Journal of
Psychopharmacology
Public Health Reports
Research Communications in Chemical Pathology and Pharmacology
Residue Reviews
Science
Science of the Total Environment
Society of Experimental Biology and Medicine, Proceedings of the
Talanta
Toxicological and Environmental Chemistry Reviews
Toxicology
Toxicology and Applied Pharmacology
Toxicology and Environmental Health, Journal of
Toxicology Letters
Xenobiotica

[a] The journals actually searched during this program include all those referenced by the Chemical Abstract Data Base, Table 1-1 is only a small fraction.

TABLE 1-2. TARGET COMPOUNDS

Compound	CAS Number	Found in the Search
Organic Acids		
Formic acid	64-18-6	
Sodium *p*-chlorobenzoate	74-11-3	
Cacodylic acid	75-60-5	X[c]
Trichloroacetic acid[a]	76-03-9	X
Propionic acid	79-09-4	X
Acrylic acid	79-10-7	
Chloroacetic acid	79-11-8	X
4,4'-Diamino-2,2'-stilbene-disulfonic acid (Amsonic acid)	81-11-8	
2,4,5-Trichlorophenoxy acetic acid[a]	93-76-5	X
2-Chloro-4-nitro benzoic acid	99-60-5	
Maleic acid	110-16-7	
Chlorendic acid	115-28-6	
Anthranilic acid	118-92-3	
Sodium acetate	127-09-3	
Nitrilotriacetic acid	139-13-9	
Nabam	142-59-6	
Oxalic acid[a]	144-62-7	X
Trisodium ethylenediamine tetraacetate	150-38-9	
Tannic acid	1401-55-4	
Hydrofluoric acid	7664-39-3	
2,4-Dichlorophenoxy acetic acid	94-75-7	
2,3-Dichlorophenoxy acetic acid	2976-74-1	
3,4-Dichlorophenoxy acetic acid	588-22-6	
Sodium xylene sulfonate	25321-41-9	
2-Methoxy-3,6-dichlorobenzoic acid	1918-00-9	X
4-Amino-3,5,6-trichoropicolinic acid	1918-12-1	X
Amines		
1-Amino-2,4-dibromoanthroquinone	81-49-2	
2-Methylaziridine	75-55-8	
Aniline hydrochloride	142-04-1	
Aniline	62-53-3	
p-Aminoazobenzene	60-09-3	
2,6-Xylidene	87-62-7	
3,3'-Dichlorobenzidine	91-94-1	X
Benzidine	92-87-5	
5-Chloro-*o*-toluidine	95-70-4	
o-Aminoazotoluene	97-56-3	
2-Nitro-*p*-phenylenediamine	5307-15-2	
4-Nitro-*o*-phenylenediamine	99-56-9	
p-Nitroaniline	100-01-6	
4,4'-Methylene bis(2-chloroaniline)	101-14-4	
Ammonia[a]	7664-41-7	X
o-Toluidine	95-53-4	X
m-Toluidine	108-44-1	X

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Amines (continued)		
p-Toluidine	106-49-0	X
Tocainide	41708-72-9	X
N-Phenyl-p-phenylenediamine	101-54-2	
4,4'-Diaminodiphenylmethane	101-77-9	
4,4'-Oxydianiline	101-80-4	
1,3-Diphenylguanidine	102-06-7	
ε-Caprolactam	105-60-2	
Propylamine	107-10-08	
p-Chloroaniline	108-42-9	
m-Chloroaniline	106-47-8	
Melamine	108-78-1	
Pyridine	110-86-1	
2-Aminoanthroquinone	117-79-3	
3,3'-Dimethoxybenzidine	119-90-4	X
p-Cresidine	120-71-8	
2-Amino-5-nitrothiazole	121-66-4	
1,6-Hexanediamine	124-09-4	
Dimethylamine	124-40-3	X
3-Amino-9-ethylcarbazole hydrochloride	132-31-1	
N-Phenyl-2-naphthylamine	135-88-6	
2,4-Diaminophenol dihydrochloride	137-09-7	
4,4'-Thiodianiline	139-65-1	
Dipropylamine	142-84-7	
1,2-Dihydro-2,2,4-trimethyl quinoline	147-47-7	
Ethylenediamine dihydrochloride	333-18-6	
2,5-Toluidine hydrochloride	636-21-5	
N-(1-Naphthyl)ethylenediamine dihydrochloride	1465-25-4	
Trifluralin	1582-09-8	
2-Bromo-4,6-dinitroaniline	1817-73-8	
Atrazine	1912-24-9	
4-Chloro-m-phenylenediamine	5131-60-2	
4-Chloro-o-phenylenediamine	95-83-0	
2,4-Diaminoanisole sulfate	39156-41-7	X
2,5-Diaminoanisole sulfate	42909-20-5	X
2,6-Diaminoanisole sulfate	102-51-2	X
Organometallics		
Triphenyltin hydroxide	76-87-9	
Zineb	142-14-3	
Maneb	301-03-1	
Lead acetate	301-04-2	
Dibutyltin diacetate	1067-33-0	
Titanocene dichloride	1271-19-8	
Lead subacetate	1335-32-6	
Ferbam	14484-64-1	
Lead dimethyltriocarbonate	19010-66-3	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Quarternary Ammonium Salts		
Ammonium acetate	631-61-8	
Ammonium sulfate	7783-20-2	
Nitrosoamines		
Nitrosopyrrolidine	930-55-2	X
N-Nitrosodimethylamine[a]	62-75-9	X
N-Nitrosodiethylamine[a]	55-18-5	X
N-Nitrosomorpholine	59-89-2	
N-Nitrosodiphenylamine	86-30-6	
p-Nitrosodiphenylamine	156-10-5	
N-Nitrosodipropyl amine	621-64-7	
Nitrosomethylurea	684-93-5	
Nitrosoethylurea	759-73-9	
N-Nitrosodi-n-butylamine	924-16-3	X
N-Nitrosopiperidine	100-75-4	X
Phenols		
Hydroquinone	123-31-9	
o-Resorcinol	108-46-13	X
m-Catechol	120-80-9	X
2,4-Dimethylphenol	105-67-9	
Bisphenol A	80-05-7	
4,6-Dinitro-o-cresol	534-52-1	
4-Chloro-3-methylphenol	59-50-7	
2-Chloro-6-methylphenol	615-74-7	
4-Chloro-2-methylphenol	1570-64-5	
2,3,4,6-Tetrachlorophenol[a]	58-90-2	X
2,3,4,5-Tetrachlorophenol[a]	4901-51-3	X
Phenol[a]	108-95-2	X
Cresol[a]	1319-77-3	X
4-Chlorophenol[a]	108-43-0	X
2-Chlorophenol[a]	95-57-8	X
3-Chlorophenol[a]	106-48-9	X
2,4-Dichlorophenol[a]	120-83-2	X
2,4,6-Trichlorophenol[a]	88-06-2	X
2,4,5-Trichlorophenol[a]	95-95-4	X
Pentachlorophenol[a]	87-86-5	X
2-Nitrophenol[a]	88-75-5	X
4-Nitrophenol[a]	100-02-07	X
α-Naphthol	90-15-3	
2,4-Dinitrophenol	51-28-5	
2-Amino-4-nitrophenol	99-57-0	
4-Amino-2-nitrophenol	119-34-6	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Miscellaneous Nitrogen-Containing Compounds		
Diuron	330-54-1	
N,N-Dimethylformamide	68-12-2	
Hexamethylphosphoramide	680-31-9	
Azodiacarbonamid	123-77-3	
Hydrazine	302-01-2	
Captan	133-06-2	X
Dioxacarb	6988-21-2	X
1-Phenyl-2-thiourea	103-85-5	
Trimethylthiourea	2489-77-2	
Ethylene thiourea	96-45-7	
Carbofuran	1563-66-2	
Vernolate	1929-77-7	
Methylcarbamate	598-55-0	
N-Propylcarbamate	627-12-3	
1,1-Dimethylhydrazine	57-14-7	
1,2-Diphenylhydrazine	122-66-7	
1-Phenyl-3-methyl-5-pyrazolone	89-25-8	
1-H Benzotriazole	95-14-7	
p-Quinone dioxime	105-11-3	
Semicarbazide hydrochloride	563-41-7	
1,3-Dichloro-5,5-dimethyl hydantoin	118-52-5	
Disulfiram	97-77-8	
2,4-Toluene diisocyanate	584-84-9	
Urethane	51-79-6	
6-Nitrobenzimidazole	94-52-0	
Chloroxuron	1982-47-4	
Alachlor	15972-60-8	
Carbaryl	63-25-2	X
2-Chlorallyl diethylthiocarbamate (CDEC)	95-06-7	
N,N'-Diethylthiourea	105-55-5	
Cyanogen chloride	506-77-4	
Acrylamide	79-06-1	
N,N'-Dimethylurea	96-31-1	
Acetamide	60-35-5	
Ethyleneimine	151-56-4	
2-Mercaptobenzothiazole	583-39-1	
Bromacil	314-40-9	
Allyl isothiocyanate	57-06-7	
Methomyl	16752-77-5	
Cupferron	135-20-6	
N,N'-Bis(1,4-dimethylpentyl)-p-phenylene diamine	3081-14-9	
Nitrofuran	27194-24-7	
N,N-Dipropylurea	619-35-2	
N,N'-Dipropylurea	623-95-0	
Methylene bis(4-phenol-2-isocyanate)	10031-75-1	
Ammonium cyanide	12211-52-8	
Pronamide	23950-58-5	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Alcohols		
2,3-Dibromo-1-propanol	96-13-9	
α-Hydroxyacetaldehyde	141-46-8	
2-Propanol	67-63-0	X
Glycidol	556-52-5	
1,4-Dichloro-2-hydroxybutane	2419-74-1	
1,2-Dihydroxybutene-2	50317-11-8	
Chloral hydrate	302-17-02	
Methanol[a]	67-56-1	X
Ethylene glycol[a]	107-21-1	X
1,2,3-Trihydroxybutane	4435-50-1	
Diacetone alcohol	132-42-2	
Benzoin	119-53-9	
Benzyl alcohol	100-51-6	
Diethylene glycol	111-46-6	
Ethylene glycol monoethyl ether	110-80-5	
Triethylene glycol	112-27-6	
Diethylene glycol monobutyl ether	112-34-5	
t-Butyl alcohol	75-65-0	X
n-Butyl alcohol	71-36-3	
Isobutyl alcohol	78-92-2	X
Aldehydes		
Formaldehyde	50-00-0	
Acetaldehyde[a]	75-07-0	X
Phosgene	75-44-5	
2-Furfuraldehyde	98-01-1	
Acrolein	107-02-8	
Chloroacetaldehyde	107-20-0	
Glutaraldehyde	111-30-8	
Propionaldehyde	123-38-6	
Paraldehyde	123-63-7	
Butyraldehyde	123-72-8	
Isobutyraldehyde	78-84-2	
Endrin aldehyde	7421-93-4	
Phosphate Esters		
Parathion[a]	56-38-2	X
Tricresyl phosphate	78-30-8	
Tris(2-ethylhexyl)phosphate	78-42-2	
Tetraethylpyrophosphate (TEPP)	107-49-3	
Tris(2-chloroethyl)phosphate	115-96-8	
Malathion	121-75-5	X
O,O,O-Triethyl phosphorthioate	126-68-1	
Methyl parathion[a]	298-00-0	X
Phorate	298-02-2	
Disulfoton	298-04-4	
O,O-Diethyl phosphorodithioate	298-06-6	X

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Phosphate Esters (continued)		
Diazinon	333-41-5	
Dichlorvos	62-73-7	X
Trimethyl phosphate	512-56-1	
Methyl paraoxon	950-35-6	
Dimethyl phosphorothioic acid[a]	1112-38-5	X
O,O-Dimethyl phosphorodithioate[a]	756-80-9	X
O,O-Dimethyl phosphorothioate[a]	2465-65-8	X
Sulfate Esters		
Diethyl sulfate	64-67-5	
Endosulfan[a]	115-29-7	X
Endosulfan sulfate	1031-07-8	X
Acid Anhydrides		
Succinic anhydride	108-30-5	
Trimellitic anhydride	552-30-7	
Phthalic anhydride	85-44-9	
Carboxylic Acid Esters		
Ethyl acetate	141-78-6	
Di-*n*-butylphthalate[a]	84-74-2	X
Di(2-ethylhexyl)phthalate[a]	117-81-7	X
n-Butylbenzyl phthalate	85-68-7	
Di-*n*-octyl phthalate	117-84-0	
Dimethyl phthalate	131-11-3	
Propyl gallate	121-79-9	
Diethyl phthalate	84-66-2	
Diallyl phthalate	131-17-9	
Dimethyl terphthalate	120-61-6	
Maleic anhydride	108-31-6	
Methyl methacrylate	80-62-6	
Diethyl maleate	141-05-9	
Diethyl carbonate	105-58-8	
Ethyl acrylate	140-88-5	
Di(2-ethylhexyl)adipate	103-23-1	
Nitro Compounds		
1,3,5-Trinitrobenzene	99-35-4	
1,2,3,4-Tetrachloro-5-nitrobenzene	879-39-0	
1,2,3,5-Tetrachloro-4-nitrobenzene	3714-62-3	
1,2,4,5-Tetrachloro-3-nitrobenzene	117-18-0	
m-Dinitrobenzene	99-65-0	
o-Dinitrobenzene	528-29-0	
p-Dinitrobenzene	100-25-4	
5-Nitro-*o*-anisidine	99-59-2	
β-Bromo-β-nitrostyrene	7166-19-0	
2-Nitropropane	79-46-9	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Nitro Compounds (continued)		
1-Nitropropane	108-03-2	
o-Nitrotoluene	88-72-2	
m-Nitrotoluene	99-08-1	
p-Nitrotoluene	99-99-0	
o-Nitroanisole	91-23-6	
2,4-Dinitrotoluene	121-14-2	
2,6-Dinitrotoluene	606-20-2	
Nitrobenzene	98-95-3	
Ketones		
Sulfolene	77-79-2	
Isophorone	78-59-1	
Methyl ethyl ketone	78-93-3	
Micheler's ketone	90-94-8	
γ-Butyrolactone	96-48-0	
Acetophenone	98-86-2	
Cyclohexanone	108-94-1	
p-Quinone	121-79-9	
1,2-Naphthoquinone	524-42-5	
1,4-Naphthoquinone	130-15-14	
2-Chloroacetophenone	532-27-4	
Methyl-*n*-butyl ketone	591-78-6	
2-Butanone peroxide	1338-23-4	
Methyl isobutyl ketone	108-10-1	X
Ethers		
Dieldrin[a]	60-57-1	X
Ethylene oxide	75-21-8	
Propylene oxide	75-56-9	
Styrene oxide	96-09-3	
4-Bromophenyl phenyl ether	101-55-3	
Vinyl cyclohexene dioxide	106-87-6	
Epichlorohydrin	106-89-8	
Allyl glycidyl ether	106-92-3	
Ethylene chlorohydrin	107-07-3	
Chloromethyl methyl ether	107-30-2	
Bis(2-chloro-1-methylethyl)ether	108-60-1	
Tetrahydrofuran	109-99-9	
2-Chloroethyl vinyl ether	110-75-8	
Bis(2-chloroethyl) ether	111-44-4	
Bis(2-chloroethoxy) methane	111-91-1	
Bis(chloromethyl) ether	542-88-1	
Decabromodiphenyl oxide	1163-19-5	
2,3,7,8-TCDD	1746-01-6	X
Di(1-chloromethyl-2-propenyl) ether	3970-20-5	
4-Chlorophenyl phenyl ether	7005-72-3	
Tetrachloroethylene oxide	16650-10-5	
Trichloroethylene oxide	16967-79-6	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Ethers (continued)		
p-Dioxane[a]	123-91-1	X
Heptachloro epoxide[a]	1204-57-3	X
Endrin	72-20-8	X
Octachloro epoxide[a]	26880-48-8	X
Nitriles		
Malononitrile	109-77-3	
Acetone cyanohydrin	75-86-5	
Nicotinonitrile	100-54-9	
Acrylonitrile	107-13-1	
Fumaronitrile	764-42-1	
Succinonitrile	110-61-2	
Methacrylonitrile	126-98-7	
Acetonitrile	75-05-8	
Hydrogen cyanide[a]	57-12-5	X
Sulfur Compounds		
Carbon disulfide	75-15-0	X
Dimethyl disulfide	624-92-0	X
Propyl mercaptan	29255-31-0	
Ethyl mercaptan	75-08-1	
Chloroethyl ethyl sulfide	693-07-2	
Halogenated Aromatic Hydrocarbons		
PCBs[a]	[b]	X
Chlorodiphenyl ethylene	13700-82-8	
Chlorodiphenyl ethane	61965-78-4	
Monochlorodiphenyl ethylene	4541-89-31	
Dichlorodiphenyl ethane	2387-16-8	
1,2,4,5-Tetrachlorobenzene	95-94-3	
p,*p*'-DDT[a]	50-29-3	X
o,*p*'-DDT[a]	789-02-6	X
p,*p*'-DDE[a]	72-55-9	X
p,*p*'-DDD[a]	72-54-8	X
Hexachlorobenzene[a]	118-74-1	X
Chlorobenzene[a]	108-90-7	X
o-Dichlorobenzene[a]	95-50-1	X
m-Dichlorobenzene[a]	541-73-1	X
p-Dichlorobenzene[a]	106-46-7	X
Pentachlorobenzene	608-93-5	
Pentachlorotoluene	877-11-2	
1,2,4-Trichlorobenzene	120-82-1	
m-Chlorotoluene	108-41-8	
o-Chlorotoluene	95-49-8	
p-Chlorotoluene	106-43-4	
Trichlorotoluene	98-07-7	
Benzyl chloride	100-44-7	
2,4-Dichlorotoluene	95-73-8	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Halogenated Aromatic Hydrocarbons		
3,5-Dichlorotoluene	25186-47-4	
2,4,6-Trichlorotoluene	23749-65-7	
1,2,4,5-Tetrachlorotoluene	1006-31-1	
1,2,3,4-Tetrachlorotoluene	1006-32-2	
2-Chloronaphthalene	91-58-7	
1,3,5-Tri-α-chloromethyl benzene	17299-97-7	
Trichlorodiphenyl ethane	2971-22-4	
Biphenyl Aroclor derivatives	1336-36-3	
Aroclor 1221	11-042-82	
Aroclor 1232	11-411-65	
Aroclor 1242	534-692-19	
Aroclor 1248	126-722-96	
Aroclor 1254	110-976-91	
Aroclor 1260	110-968-25	
2,3,5-Tri-α-dichloromethyl benzene	56682-87-2	
1,2-Chloronitrobenzene	88-73-3	
1,3-Chloronitrobenzene	121-73-3	
1,4-Chloronitrobenzene	100-00-5	
Halogenated Aliphatic Hydrocarbons		
1,2,2,3,3,4-Hexachlorobutane	1573-57-5	
1,1,2,2,3,4-Hexachlorobutane	2431-55-2	
4-Chlorobutyne-1	51908-64-6	
1-Chlorobutyne-1	62981-74-2	
1,4-Dichlorobutene-1	31423-92-4	
3,4-Dichlorobutene-1	7013-11-8	
1,1,2,3-Tetrachloropropene	20589-85-9	
1,1,2-Trichloropropene	21400-25-9	
1,1,3-Trichloropropene	2567-18-8	
1,2,3-Trichloropropene	96-19-5	
2,3,3-Trichloropropene	37077-84-2	
1,1,2,2,3,4,4-Heptachlorobutane	34973-41-6	
1,1-Dichlorobutadiene-1,2	6061-06-9	
1,4-Dichlorobutadiene-1,3	3588-12-3	
1,4-Dichlorobutyne-1	8201-10-3	
1,6-Dichlorohexadiene-1,5	67546-51-4	
1-Chloropentene-1	21450-13-5	
2-Chloropentene-1	42131-85-1	
3-Chloropentene-1	24356-00-1	
1-Chloropentene-2	10071-60-0	
2-Chloropentene-2	67747-70-0	
3-Chloropentene-2	26423-61-0	
4-Chloropentene-2	1458-99-7	
5-Chloropentene-2	16435-50-0	
1-Chlorohexatriene-1,3,5	2196-26-1	
2-Chlorohexatriene-1,3,5	4891-37-6	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Halogenated Aliphatic Hydrocarbons (continued)		
3-Chlorohexatriene-1,3,5	2196-26-0	
2,5-Dimethyl-1,1,6,6-tetrachlorohexadiene-1,5	19792-19-4	
2,7-Dichlorooctadiene-2,6	17817-99-1	
2,8-Dichlorooctadiene-1,4	72161-23-0	
3,5-Dichloro-2,4-dimethylhexadiene-1,3	78592-51-5	
1,3-Dichloro-2,4-dimethylhexadiene-2,4	78592-20-8	
1,1,3-Trichlorooctane	4905-80-0	
2,4,4-Trichlorooctane	63616-37-5	
1,2,3,3,3-Pentachloropropene	7614-75-7	
1,1,2,3,3-Pentachloropropene	1600-37-9	
1,3-Dichloroallene	927-75-3	
Octachlorobutene-1	39388-41-5	
1-Chloropentane	543-59-9	
Chlordane[a]	57-74-9	X
Chlorodene	56534-03-3	
Aldrin[a]	309-00-2	X
2-Chloroprene	557-98-2	
3-Chloroprene	107-05-1	
1,1,1,6,6,6-Hexachlorohexane	30353-57-2	
1,3,3,4,4,6-Hexachlorohexane	58468-00-1	
1,1,4,4,5,6-Hexachlorohexene-1	60870-81-7	
1,5,5,6,6,6-Hexachlorohexene-2	68110-46-3	
1,2,3,4,5,6-Hexachlorohexene-3	1725-74-2	
Tetrachloromethane[a]	56-23-5	X
Dichlorodifluoromethane[a]	75-71-8	X
Trichlorofluoromethane[a]	75-69-4	X
Dibromochloromethane	124-48-1	X
Bromodichloromethane	75-27-4	X
Chloroform[a]	67-66-3	X
Dichloromethane[a]	75-09-2	X
Tetrachloroethene[a]	127-18-4	X
Trichloroethene[a]	79-01-6	X
Trichlorotrifluoroethane[a]	354-58-5	X
1,1-Dichloroethene	75-35-4	X
1,2-Trans-vinylidene chloride[a]	540-59-0	X
Vinyl chloride	75-01-4	X
1,1,1-Trichloroethane[a]	71-55-6	
1,1,2-Trichloroethane	79-00-5	
1,1-Dichloroethane	75-34-3	
1,2-Dichloroethane	107-06-2	
α-Hexachlorocyclohexane[a]	319-84-6	X
β-Hexachlorocyclohexane[a]	319-85-7	X
δ-Hexachlorocyclohexane[a]	319-86-8	X
γ-Hexachlorocyclohexane (Lindane)	58-89-9	X
1,4-Dichlorobutane	110-56-5	
1,6-Dichlorohexatriene-1,3,5	69645-07-4	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Halogenated Aliphatic Hydrocarbons (continued)		
n-Butyl chloride	109-69-3	
Hexachlorocyclopentadiene	77-47-4	
Chlorobutene	591-97-9	
1,2,4-Trichlorobutane	1790-22-3	
1,3,3-Trichlorobutane	15187-71-0	
1,3-Dichloro-2-methyl propene	3375-22-2	
4-Chlorobutene-1	927-73-1	
1-Chloro-2-methyl propene	513-37-1	
1,2,3,4-Tetrachlorobutane	3405-32-1	
2-Chloromethyl-3-chloropropene	1871-57-4	
1,1,2,3,4-Pentachlorobutane	27303-96-4	
1,2,4-Trichlorobutene-2	2431-54-1	
1,1,4-Trichlorobutene-2	41601-59-6	
2-Chloromethyl-3,3-Dichloropropene	60845-51-4	
2-Chloromethyl-1,3-Dichloropropene	13245-65-3	
2-Chlorobutadiene	126-99-8	
trans-Nonachlor[a]	39765-80-5	X
1,1,2,2-Tetrachloroethene[a]	127-18-4	X
1,2-Dibromoethane	106-93-4	X
1-Chlorobutadiene-1,2	627-23-6	
3-Chlorobutadiene-1,2	34581-41-4	
1,1,1,2,2,3,3,4,4-Nonachlorobutane	21483-62-5	
1,1,2-Trichlorobutadiene-1,3	2852-07-5	
1,1,4-Trichlorobutadiene-1,2	58679-08-6	
1,1,4-Trichlorobutadiene-1,3	2984-41-0	
1,1,2,2,3,3,4,4-Octachlorobutane	20338-26-5	
1,1,1,2,3,4,4,4-Octachlorobutane	18791-19-0	
1,1,1,2,3,4,4,4-Octachlorobutane	32694-76-1	
Perchlorobutane	6820-74-2	
Hexachlorobutadiene-1,3	87-68-3	
Hexachlorobutadiene-1,2	56827-79-3	
2-Chloropropane	75-29-6	
1,2-Dichloropropane	78-87-5	
1,2,3-Trichloropropane	96-18-4	
Allyl chloride	107-05-1	
1,3-Dichloropropene	542-75-6	
1-Chloropropyne	7747-84-4	
3-Chloroallene	1024-65-7	
Hexachlorophene	1888-71-7	
Chloroethane	75-00-3	
Ethyl bromide	74-96-4	
Ethylene dibromide	106-93-4	X
1,1,2,2-Tetrachloroethane	79-34-5	
Pentachloroethane	76-01-7	
1,1,2,2-Tetrachloroethane	79-34-5	
Perchloroethylane	67-72-1	
Hexachloroethane	67-72-1	
Methyliodide	74-88-4	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Halogenated Aliphatic Hydrocarbons (continued)		
Chloromethane	74-87-3	
Bromomethane	74-83-9	
Tribromomethane	75-25-2	
Toxaphene	8001-35-2	
Heptachlor	76-44-8	
4-(1,2-Dichloroethyl)-1,2-dichlorocyclohexane	51962-63	
2,5-Dimethylhexatriene-1,3,5	4916-63-6	
2,5-Dichlorohexane	13275-11-8	
1,2-Dichloro-1,2-divinyl cyclobutane	14112-00-6	
Fluorocarbons	b	X
Polynuclear Aromatic Hydrocarbons (PNA)		
Benzo[o]fluoranthene	205-99-2	
Benzo[j]fluoranthene	205-82-3	
Benzo[k]fluoranthene	207-08-9	X
Benzo[e]pyrene	192-97-2	X
1,1-Diphenylethylene	530-48-3	
Benzo[a]anthracene	56-55-3	X
Fluoroanthene	206-44-0	
Benzo[ghi]perylene	191-24-2	X
Benzo[b]fluoranthene	205-99-2	X
Ideno[1,2,3-cd]pyrene	135-39-5	
Dibenzo[ah]anthracene	53-70-3	X
Benzo[a]pyrene	50-32-8	X
Chrysene	218-01-9	
Pyrene	129-00-0	X
Anthracene	120-12-7	X
Phenanthrene	85-01-8	
Fluorene	86-73-7	
Acenaphthene	83-32-9	
Acenaphthalene	208-96-8	
Naphthalene	91-20-3	
1,1-Biphenyl	92-52-4	X
Hydrocarbons		
Propylene	115-07-1	
o-Xylene	95-476	
m-Xylene	108-38-3	X
p-Xylene[a]	106-42-3	X
1,3-Butadiene	106-99-0	
Cyclopentadiene	77-73-6	
α-Methylstyrene	98-83-9	
Dicyclopentadiene	77-73-6	
Diphenylethane	103-29-7	
Diphenylacetylene	501-65-5	
1,3,5-Triethylbenzene	102-25-0	
Dimethylhexadiene-2,4	764-13-6	

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Hydrocarbons (continued)		
1,2-Divinyl cyclobutane	2422-85-7	
2,3-Dimethylbutadiene	513-81-5	
Hexadiene-1,5	492-42-7	
Hexatriene-1,3,5	2235-12-3	
4-Vinyl-1-cyclohexene	100-40-3	
Methyl allene	590-19-2	
Allene	463-49-0	
Mesitylene	108-67-8	
Cumene	98-82-8	
Ethylbenzene	100-41-4	X
Styrene[a]	100-42-5	X
Toluene[a]	108-88-3	X
n-Hexane[a]	110-54-3	X
Cyclohexane[a]	110-82-7	X
Benzene	71-43-2	
Pentadiene	78-79-5	
Ethyl acetylene	107-00-6	
Bicycloheptadiene	121-46-0	
Miscellaneous		
3-Hydroxy-4-chlorobutene-1	141-75-3	
Acetyl chloride	75-35-5	
Dimethyl dithiophosphoric acid	1112-38-5	
Metals		
Aluminum salts[a]	b	X
Antimony[a]	7440-36-0	X
Antimony pentachloride	7647-18-9	
Antimony trichloride	7440-36-0	
Arsenic[a]	7440-38-2	X
Barium carbonate	7440-39-3	X
Barium chloride	10361-37-2	X
Cadmium[a]	7440-43-9	X
Cadmium chloride	10108-64-2	X
Cadmium sulfate	10124-36-4	X
Cadmium sulfide	1306-23-6	
Calcium chromate	13265-19-0	X
Chromium[a]	7440-47-3	X
Copper salts	b	X
Lead[a]	7439-92-1	X
Manganese[a]	7439-96-5	X
Manganese dioxide	1313-13-9	
Magnesium hydroxide	7439-95-4	X
Mercuric chloride	7487-94-7	
Mercury	7439-97-6	X
Molybdenum trioxide	7439-98-7	X
Nickel[a]	7440-02-0	X

(continued)

TABLE 1-2. (continued)

Compound	CAS Number	Found in the Search
Metals (continued)		
Nickel carbonyl	13463-39-3	
Nickel oxide	1313-99-1	
Palladium	7440-05-3	X
Silver	7440-22-4	X
Sodium fluoride	16984-48-8	X
Stannous chloride	7772-99-8	X
Strontium	7440-24-6	X
Strontium chromate	7789-06-2	
Tin[a]	7440-31-5	X
Titanium	7440-32-6	X
Tungsten	7440-33-7	X
Vanadium	7440-62-2	X
Zinc	7440-66-6	X

[a] Identified in one of the biological matrices of interest and citations reported information on analysis method.

[b] Multiple CAS numbers.

[c] X indicates it was found in the search.

TABLE 1-3. HIERARCHICAL SCHEME FOR ORGANIC CHEMICALS

1. Phosphoric Acids
2. Organometallic Acids
3. Carboxylic Acids
4. Basic Nitrogen Compounds
5. Nitrosoamines
6. Phenols
7. Nitrogen Containing Compounds
8. Alcohols
9. Aldehydes
10. Sulfate Esters
11. Phosphate Esters
12. Carboxylic Acid Esters
13. Ketones
14. Sulfur Compounds
15. Ethers
16. Nitriles
17. Halogenated Aliphatics
18. Halogenated Aromatics
19. Hydrocarbons

functional group with a greater influence on aqueous solubility, boiling point, extraction behavior, and chromatographic properties. If a compound contains more than one functional group, it is placed in the highest chemical classification. Because this classification is based on the physical/chemical properties, it should reflect analytical behavior of many of the compounds and aid in the overall evaluation process. Chemicals found in the search are shown in Table 1-2; however, other chemicals which were not initially searched but later found during the review of the CAS data base are mentioned in the specific sections.

Literature Compilation

Information from the data base "Chemicals Identified in Human Biological Media" on (1) literature referenced, (2) chemicals analyzed, (3) biological matrix, and (4) analytical technique was entered and stored on computer files. These data were retrieved according to analytical method. Under each analytical method, citations referring to that method were listed according to chemical class, specific chemical, and biological matrix. The "Chemical Abstract Data Base" was searched using the compounds listed in Table 1-2; they were cross-referenced by CAS number with the human biological matrices of interest and with the techniques most likely to be applicable. This information was used for sorting retrieved literature citations and disseminating articles to members of the advisory committee who were responsible for the preparation of this review. This information was also used to match analytes with biological matrix in order to determine the feasibility of correlating analytical method with biological matrix and with chemical characteristics.

Relevant articles which report the use of an analytical technique to measure any of the target chemicals in the six biological matrices of interest were obtained. This information is compiled in Table 1-4. All analytical techniques cited by both data bases have been included in this table. Only those methods which had at least three articles cited were included in the review.

TABLE 1-4. RELEVANT CITATIONS IN BOTH DATA BASES

Analytical Method	Number of Citations
Atomic absorption spectrometry	308
Electrochemical methods	21
Column chromatography[a]	18
Emission spectroscopy	17
Gas chromatography	84
Mass spectrometry	43
High-performance liquid chromatography	22
Neutron activation analysis	61
Nuclear magnetic resonance	3
Proton induced x-ray spectroscopy	22
Thin-layer chromatography	8
X-ray spectroscopy	9
Spectrophotometry (colorimetry)	42

[a] Included in the section on Preparation Methods for Organic Analysis.

Literature Acquisition

During acquisition of literature citations, greater than 95% of all citations identified as relevant, i.e., those which reported measuring any of the target chemicals in the six biological matrices, were retrieved. Missing citations were not retrieved for several reasons:

(a) the article was in a foreign language which could not be translated easily;

(b) the reference in the data base was incorrect; or

(c) a source of the article could not be located.

Written Evaluation

This Final Report was written to evaluate each analytical method. To provide consistency, all evaluations were written using a single format. The format for each section is listed in Table 1-5. The evaluation for each method includes sections on instrumentation and sample preparation methods. Since the review was not to be limited to the citations in the data base, much of the information on analytical instrumentation was taken from review articles, reference texts, or our working knowledge of the techniques. Information on sample preparation methods were generally restricted to the citations in the data base.

The section on method/analyte correlation parameters attempts to suggest physical or chemical properties of analytes which could be used to predict the applicability of the given method to the analysis of that chemical in a specific biological matrix.

TABLE 1-5. FORMAT FOR REPORT

SECTION: (ANALYTICAL METHOD)

INSTRUMENTATION

Principle of the Technique
Chemicals Analyzed
Methods of Quantitation
Limit of Detection
Precision
Accuracy
Simplicity, Cost, and Sample Throughput

SAMPLE PREPARATION METHODS[a]

Preparation Method
- Principle of the Technique--
- Biological Matrices--
- Chemicals Analyzed/Physical-Chemical Properties--
- Method Interferences--
- Concentration Factor/LOD--
- Recovery Data/Quantitation Method--
- Precision--
- Simplicity, Cost, and Sample Throughput--

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

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[a] Preparation methods for organic compounds are included in a separate section.

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SECTION 2

SUMMARY AND CONCLUSIONS

Methods and procedures which were cited in both data bases for the identification and quantitation of the target chemicals in the six human biological matrices are summarized in Table 2-1. Broad generalizations are difficult to formulate due to the complexity of compounds, matrices, and methods reviewed; reference to the sections on individual methods is recommended. Table 2-1 provides a noncritical survey of the analytes for each method in the data base. A brief summary of the advantages and disadvantages of each of the methods reviewed follows.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

Atomic absorption spectrophotometry has been the method of choice for many investigators who require a highly specific, low-cost technique with ultratrace detection limits. The advantages of this single-element method have been known for the past 15 years, but the use of modern instrumentation has produced analytical data of greater reproducibility and has resulted in increased sample throughput. One of the best-documented environmental measurements (lead in whole blood) is usually performed by atomic absorption methods.

The biggest disadvantage of this technique is not the inherent limitations of the method with respect to analyte specificity and sensitivity, but the frequent difficulty in reducing the effect of matrix interferences to an acceptable level. A variety of digestion conditions have been developed which in combination with deuterium background correction and/or chelation-extraction, such as APDC-MIBK, have been successful in reducing matrix effects and form the basis for most atomic absorption procedures.

TABLE 2-1. APPLICATION OF ANALYTICAL TECHNIQUE TO SELECTED CHEMICALS

Compounds	Analytical Technique[a]										
	AA	NA	EC	XS	ES	COL	GC	LC	MS	NMR	XRS
Organic Acids											
Cacodylic acid							X				
Trichloroacetic acid							X				
Chloroacetic acid						X	X				
2,4,5-Trichlorophenoxy acetic acid							X				
Oxalic acid							X	X			
2-Methoxy-3,6-dichlorobenzoic acid							X				
4-Amino-3,5,6-trichloropicolinic acid							X				
Propionic acid								X			
Amines											
Benzidine								X			
3,3'-Dichlorobenzidine							X	X			
Ammonia						X					
o-Toluidine							X				
m-Toluidine							X				
p-Toluidine							X				
Tocainide							X				
Dimethyl amine									X		
2,4-Diaminoanisole sulf[illegible]											
2,5-Diaminoanisole sulfate											
2,6-Diaminoanisole sulfate											
3,3'-Dimethoxybenzidine								X			
Nitrosoamines											
Nitrosopyrrolidine							X				
N-Nitrosodimethylamine							X				
N-Nitrosodiethylamine							X				
N-Nitrosodi-*n*-butylamine							X				
N-Nitrosopiperidine							X				
Phenols											
m-Catechol								X			
o-Resorcinol								X	X		
2,3,4,6-Tetrachlorophenol								X	X		
2,3,4,5-Tetrachlorophenol								X	X		
Phenol						X	X		X		
Cresol							X		X		
4-Chlorophenol							X	X	X		
2-Chlorophenol							X	X	X		
3-Chlorophenol							X	X	X		
2,4-Dichlorophenol							X	X	X		
2,4,6-Trichlorophenol							X	X	X		
2,4,5-Trichlorophenol							X	X	X		
Pentachlorophenol							X	X	X		
2-Nitrophenol							X	X			
4-Nitrophenol							X	X			

(continued)

TABLE 2-1. (continued)

Compounds	Analytical Technique[a]										
	AA	NA	EC	XS	ES	COL	GC	LC	MS	NMR	XRS
Miscellaneous Nitrogen-Containing Compounds											
Captan							X				
Dioxacarb							X				
Carbaryl							X				
Alcohols											
2-Propanol							X			X	
Methanol						X	X		X	X	
Ethylene glycol							X				
Tert-butyl alcohol							X				
Isobutyl alcohol							X				
Trichloroethanol						X					
Aldehydes											
Acetaldehyde							X				
Phosphate Esters											
Parathion							X		X		
Malathion							X		X		
Methyl parathion							X		X		
O,O-Diethyl phosphorodithioate							X		X		
Dichlorvos							X		X		
Dimethyl phosphorothioic acid							X		X		
O,O-Dimethyl phosphorodithioate							X		X		
O,O-Dimethyl phosphorothioate							X		X		
Sulfate Esters											
Endosulfan							X		X		
Endosulfan sulfate							X		X		
Carboxylic Acid Esters											
Di-n-butylphthalate						X	X		X		
Di(2-ethylhexyl)phthalate						X	X	X	X		
Ketones											
Acetone										X	
Methyl isobutyl ketone							X				
Ethers											
Dieldrin							X	X	X		
p-Dioxane							X		X		
Heptachloro epoxide							X	X	X		
Endrin							X				
Octachloro epoxide							X	X	X		

(continued)

TABLE 2-1. (continued)

Compounds	AA	NA	EC	XS	ES	COL	GC	LC	MS	NMR	XRS
	Analytical Technique[a]										
Nitriles											
Hydrogen cyanide		X	X			X	X				
Sulfur Compounds											
Carbon disulfide							X		X		
Dimethyl disulfide							X		X		
Halogenated Aromatic Hydrocarbons											
PCBs							X	X	X		
p,*p*'-DDT							X	X	X		
o,*p*'-DDT							X	X	X		
p,*p*'-DDE							X	X			
p,*p*'-DDD							X	X			
Hexachlorobenzene							X	X			
Chlorobenzene							X	X	X		
o-Dichlorobenzene									X		
m-Dichlorobenzene							X		X		
p-Dichlorobenzene							X		X		
Tetrachlorodibenzo-*p*-dioxin								X	X		
Halogenated Aliphatic Hydrocarbons											
Chlordane							X				
Aldrin							X				
Tetrachloromethane							X		X		
Dichlorodifluoromethane							X				
Trichlorofluoromethane							X		X		
Dibromochloromethane							X				
Bromodichloromethane							X				
Chloroform							X		X		
Dichloromethane							X		X		
Tetrachloroethene						X	X		X		
Trichloroethene							X		X		
Trichlorotrifluoroethane							X		X		
1,1-Dichloroethene							X		X		
1,2-Trans-vinylidene chloride							X		X		
Vinyl chloride							X		X		
α-Hexachlorocyclohexane							X	X			
β-Hexachlorocyclohexane							X				
δ-Hexachlorocyclohexane							X				
γ-Hexachlorocyclohexane (Lindane)							X				
trans-Nonachlor							X	X	X		
1,1,2,2-Tetrachloroethene							X		X		
1,2-Dibromoethane							X		X		
Ethylene dibromide							X		X		

(continued)

TABLE 2-1. (continued)

Compounds	Analytical Technique[a]										
	AA	NA	EC	XS	ES	COL	GC	LC	MS	NMR	XRS
Polynuclear Aromatic Hydrocarbons (PNA)											
1,1-Biphenyl							X	X			
Anthracene								X			
Pyrene								X			
Benzo[e]pyrene								X			
Benzo[a]anthracene								X			
Benzo[b]fluoranthene								X			
Benzo[k]fluoranthene								X			
Benzo[a]pyrene								X			
Benzo[ghi]perylene								X			
Dibenzo[ah]anthracene								X			
Hydrocarbons											
m-Xylene							X	X	X		
p-Xylene							X	X	X		
Ethylbenzene							X		X		
Styrene							X		X		
Toluene							X	X	X		
n-Hexane							X		X		
Cyclohexane							X		X		
Miscellaneous											
Organic fluoride				X							
Metals											
Aluminum salts	X				X						
Antimony	X	X			X						
Arsenic	X	X		X	X	X				X	
Barium	X	X	X	X	X						
Cadmium	X	X	X		X	X					
Calcium	X	X		X	X	X				X	X
Chromium	X	X	X		X	X					
Copper salts	X								X		X
Lead	X		X	X		X					X
Manganese	X	X		X		X					
Magnesium	X	X		X	X	X				X	X
Mercury	X			X	X						
Molybdenum	X				X	X					X
Nickel	X	X	X	X	X						
Palladium	X										
Silver	X	X									
Sodium	X	X			X						
Selenium	X		X	X							X
Strontium	X	X									X
Tin	X		X		X						
Titanium	X	X									
Tungsten	X		X		X	X					
Vanadium	X	X				X					
Zinc	X	X	X	X	X			X			X

FOOTNOTE FOR TABLE 2-1

[a] AA - atomic absorption spectroscopy;
NA - neutron activation analysis;
EC - electrochemical methods;
XS - porton induced x-ray spectroscopy;
ES - emission spectroscopy;
COL - colorimetry;
GC - gas chromatography;
LC - liquid chromatography, column chromatography, thin layer chromatography;
MS - mass spectrometry;
NMR - nuclear magnetic resonance;
XRS - x-ray spectrscopy.

NEUTRON ACTIVATION ANALYSIS (NAA)

Neutron activation allows multielement analyses on a single sample without pretreatment. Under conditions of high neutron flux, maximum sample size ($\sim$1 g) and no interferences, NAA provides detection limits which can only be matched by flameless atomic absorption spectrophotometry. Essentially all of the elements of environmental interest (with the exception of beryllium) from nitrogen to the heavy elements can be analyzed simultaneously by this technique.

The principal limiting factor in neutron activation analyses is the presence of interferences, that is, the gamma-ray signal from the various sources not being resolved. Detection limits may increase by several orders of magnitude when interferences are present. Poorer precision and accuracy also result.

With the exception of needing access to a neutron source, NAA can be relatively simple and inexpensive with the capability of measuring a large number of elements simultaneously. When either chemical methods or extensive time resolution is required to minimize interferences; the cost, time, and labor required for analysis increase significantly.

ELECTROCHEMICAL METHODS

Ion Selective Potentiometry

The facility of ion selective electrodes in the analysis of inorganic ions is a distinct advantage of this analytical technique. Although limits of detection are typically 10^{-6} $\underline{M}$, which is somewhat higher than competing techniques, fluoride and cyanide selective electrodes have been quite successfully used in the analysis of blood and urine. Their simplicity in this regard makes them highly recommended.

Anodic Stripping Voltammetry

Anodic stripping voltammetry (ASV) has been applied to the analysis of blood, urine, and hair for a large number of metals including zinc, cadmium, lead, chromium, and copper. In as much as ASV is able to differentiate between labile and nonlabile metal species, it is clearly the method of choice when information regarding metal speciation is desirable. When total metal determinations are needed, ASV is comparable in effort and accuracy to other methods.

Cyclic Voltammetry

Cyclic voltammetry has been applied to several metal determinations and in addition some organic compounds such as p-amino-diphenylamine. Blood samples have been analyzed without further treatment. Cyclic voltammetry is an easy technique to implement and the analysis is fast. The equipment cost is relatively inexpensive compared to other instruments used in the simultaneous determination of metals. The limit of detection (LOD) is higher than the one for ASV.

EMISSION SPECTROSCOPY

A large number of metals may be determined using emission spectroscopy. The limit of detection for these metals is adequate for environmental trace analysis, and many published data were found on the use of either DC or argon plasma ionization methods (described in Section 7). However, by far, the most widely used spectroscopy technique is atomic absorption (AA) spectroscopy (described in Section 4). Because of the wealth of data on method performance and the availability of instrumentation, AA would be considered the method of first choice, but ICP may be faster, more accurate, more precise and have lower LOD.

PROTON INDUCED X-RAY EMISSION (PIXE)

Proton induced X-ray analysis may be used for multielement analysis of large number of samples. Although biological samples can be analyzed in a variety of forms, including untreated specimens, best results are achieved by digesting the organic material and placing the resulting solution on a Mylar film for irradiation. The elements normally measured for PIXE range from phosphorous ($z = 15$) to lead ($z = 82$) with limits of detection at the subnanogram level.

The method is simple, allows high sample throughput, and is currently being performed by several laboratories inexpensively.

SPECTROPHOTOMETRY (COLORIMETRY)

Spectrophotometry has the advantages of being a simple and quick procedure utilizing instrumentation which is widely available and relatively inexpensive. It is capable of determining compounds and metal complexes at the low microgram to high nanogram range with good precision (2-3%). The disadvantages of this technique are the labor intensive sample preparation steps, and the nonunique nature of the measured signal (compared to AA/ICP). This method is suitable for many analytical measurements, particularly where the operating laboratory does not have AA/ICP spectrometers. Where AA or ICP are available, they would usually be the methods of choice.

GAS CHROMATOGRAPHY

Gas chromatography is used for the separation and determination of volatile or semivolatile organic compounds. Recent developments in capillary column chromatography provide for very high resolution to give separation of complex samples. The use of specific column packings allows the determination of polar and some compounds with low volatility.

Effluent from the GC column may be analyzed using a specific (e.g., electron capture, nitrogen/phosphorus) or a nonspecific (e.g., flame ionization, thermal conductivity) detector. Limits of detection range from subpicogram to microgram depending upon the detector used and the compounds determined.

Gas chromatography is generally a simple technique for the determination of organic compounds and is used extensively on human biological extracts. Compounds which are nonvolatile (B.P. >300°C), very polar, or heat labile cannot be determined directly by this method. For these compounds, high performance liquid chromatography is generally used. Some polar and/or non-volatile compounds may be derivatized to improve gas chromatographic performance.

Most biological samples cannot be analyzed directly. Analytes are usually extracted and fractionated prior to analysis. Although GC analysis itself is a simple rapid procedure, sample preparation techniques are often complicated and time-consuming.

PREPARATION METHODS FOR ORGANIC ANALYSIS

Column chromatography has been used extensively to provide the necessary prefractionation of biological samples. This two-phase separation technique is distinguished from other fractionation methods (liquid/liquid partition, thin layer) by utilization of a stationary phase contained in a packed column bed which is continuously eluted with a liquid mobile phase. The stationary phase may be coated on a solid support or the support itself may function as the stationary phase.

Open column systems are used for the separation of one compound or class of compounds from other materials that interfere with analysis. The majority of analytes requiring fractionation are nonpolar, lipophilic compounds such as organochlorine pesticides and polychlorinated biphenyls.

Materials for open column chromatography are simple. Several samples can be processed at one time making this a rather efficient and inexpensive method.

MASS SPECTROMETRY

Mass spectrometry is a sensitive, specific but expensive technique. The various forms of mass spectrometry which can be employed complicate the question of when the technique should be used. Spark source mass spectrometry is extremely valuable in elemental analysis. Atomic absorption spectrophotometers and inductively coupled argon plasma spectrometers are substantially less expensive for these analyses; however, for a semiquantitaive scan of a broad range of elements, spark source mass spectrometry could be the method of choice. Many commercial laboratories offer spark source mass spectrometric assays as a service, so purchase would not be necessary for an occasional need.

For accurate determination of isotope ratios, there is no alternative to isotope ratio mass spectrometry. If such an assay is required, a laboratory usually specializes in this assay and, therefore, makes effective use of the instrumentation.

Although gas chromatography/mass spectrometry as a combined technique is one of the most widely used analytical methods, the decision to use the technique still requires careful thought. Gas chromatography is far more cost-effective and is usually more sensitive. However, the analysis of samples of biological media for various organic constituents by gas chromatography presents various difficulties which may require the specificity inherent in the mass spectrometric technique. If, for example, identification of a pesticide is desired for forensic or regulatory purposes, very careful use of standards during gas chromatography/mass spectrometry is required in order to give the identification of the compound a high degree of confidence. Biological samples often contain material which coelutes with a compound of interest and only the specificity of the mass spectrometric technique will allow a successful assay. When standards for the compounds of interest are not available, the compounds can be identified with a degree of confidence only by the mass spectrometric technique.

In summary, mass spectrometry should be used when its specificity is required, a less expensive technique is not adequate, or when certain types of information are available from no other source, for example, accurate isotope ratios or accurate mass measurement.

X-RAY SPECTROSCOPY

X-ray emission spectroscopy can be used in chemical analysis for both quantitative and qualitative studies. X-ray spectroscopy is applicable to the determination of most metals in multielement analysis. The technique is expensive and not widely available.

NUCLEAR MAGNETIC RESONANCE (NMR)

Nuclear magnetic resonance spectroscopy is applicable to a wide range of organic compounds, provides information on chemical structure, and can be quantified. The operation of NMR instruments is rather complex, however, no significant sample preparation is required. The equipment is very expensive and not very widely available.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography is a separation technique based on adsorption or partition process and employs a thin, flat bed of sorbent. Thin layer chromatography can be used for the separation and semi-quantitation of complex mixtures and has proven useful as a cleanup technique for analytes present in biological fluid extracts and environmental samples.

With the exception of some macromolecules, reactive compounds and volatile substances, virtually any organic compound and many inorganic ions can be determined by thin layer chromatography. The limit of detection is a function of the specific detection system utilized. Semi-quantitative thin layer chromatography is fast, simple and can be accomplished with a minimum of expensive equipment. Quantitative thin layer chromatography with modern instrumental detection systems is both accurate and sensitive, but requires rather expensive equipment.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography is a simple and standard separation technique applicable to virtually any mixture of organic compounds as well as certain ionic species. It is usually applied to complex matrices for which gas chromatography is not usable, such as matrices containing high boiling point chemicals or compounds which decompose under GC conditions. A wide variety of detectors can be interfaced to a liquid chromatography column such as ultraviolet detectors, refractive index detectors and fluorimetric detectors.

MISCELLANEOUS METHODS

During this survey, a few (less than three) reports were found dealing with the determination of compounds of interest using some miscellaneous methods. The scarcity of publications did not warrant a special section for these techniques in this review.

One such report (1) describes the evaluation of the permeability of phenolic compounds through human stratum corneum using a desorption technique. In another report (2), laser microprobe mass analysis (LAMMA) is used to determine metal ions and organic compounds in solid samples which require spacial resolution.

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SECTION 3

RECOMMENDATIONS

The goal of this research program was to evaluate methodologies for determining chemicals of interest to EPA in human biological matrices. This review included the citations listed in "Chemicals Identified in Human Biological Matrices, A Data Base" and the Chemical Abstract Data Base. Based on this extensive literature, source summary reviews have been compiled according to methodology and form the bulk of this report (Sections 4 through 17).

In addition, based on the chemical and physical characteristics of the compounds of interest and the method by which they are determined, some simple and expected correlations can be found. They are shown in Table 3-1. In Table 3-1 the compounds have been listed by type in the same manner as shown in Table 1-2 for easy cross reference. Table 3-1 also shows the matrix in which they are most commonly found and the methods which have been used for analysis.

Some general recommendations can be summarized as follows:

1. Most organic compounds found in human biological matrices can be determined by gas chromatography coupled with a variety of detectors. To select a specific detector, the compound type and the matrix in which they are found must be taken into account. Sections 10, 11 and 12 show more detailed usage of gas chromatography combined with some specific detection systems.
2. Most organic compounds of high boiling point or that decompose under gas chromatographic conditions which are found in human biological matrices can be determined by liquid chromatography coupled with a variety of detectors. Again the selection of a specific detector depends on the compound type and matrix in which they are found. Sections 11, 15 and 16 show more detailed usage of liquid chromatography and related methods.

TABLE 3-1. PHYSICO-CHEMICAL CORRELATIONS

Compound Type	Matrix	Recommended Method
Organic Acids	Blood Urine Adipose	GC/ECD[a] GC/FID[b] GC/TCD[k] HPLC COL NMR[n]
Amines	Urine Blood	GC/FID GC/MS[c] GC/TCD HPLC COL
Quaternary Ammonium Salts	N/A	N/A[d]
Nitrosamines	Blood Urine Adipose	GC/FID/TCD GC/MS
Phenols	Urine Blood Adipose	GC/FID/TCD GC/MS GC/MS HPLC COL[m]
Miscellaneous Nitrogen Containing Compounds	Urine	GC/FID/TCD GC/MS HPLC
Organometallics	Urine Blood	EC[g] ICAP AA PIXE NAA
Alcohols	Blood Urine	GC/FID GC/MS COL[m]
Aldehydes	Blood	Glass capillary GC[e] GC/FID
Phosphate Esters	Adipose	GC/NPD[f]
Sulfate Esters	Tissue	GC
Acid Anhydrides	d	d
Carboxylic Acid Esters	Blood Urine	NMR GC/ECD GC/MS HPLC COL[m]

(continued)

TABLE 3-1. (continued)

Compound Type	Matrix	Recommended Method
Ethers	Blood Adipose Urine	GC/FID GC/TCD[k] HPLC
Nitro compounds	Blood Urine	EC[g] HPLC
Ketones	Urine Blood	GC/MS
Metals	Blood Urine Hair Tissue	EC[g] ICAP[h] AA[i] PIXE[j] COL[m]
Nitriles	Blood	EC COL
Sulfur Compounds	Urine	HPLC
Halogenated Aromatic Hydrocarbons	Blood Urine Tissue Adipose	GC/MS GC/HECD[l] GC/ECD TLC
Halogenated Aliphatic Hydrocarbons	Blood Urine Adipose	GC/MS GC/HECD
Polynuclear Aromatic Hydrocarbons (PAH)	Adipose Tissue	HPLC
Hydrocarbons	Adipose Urine	GC/FID TLC HPLC GC/MS

[a] Gas chromatography/electron capture detector.

[b] Gas chromatography/flame ionization detector.

[c] Gas chromatography/mass spectrometry.

[d] Not available from the searched literature.

[e] Glass capillary gas chromatography.

[f] Gas chromatography/nitrogen phosphorus detector.

[g] Electrochemical methods.

[h] Inductively coupled argon plasma.

[i] Atomic absorption spectrometry.

[j] Proton induced x-ray spectroscopy.

[k] Gas chromatography/thermal conductivity detector.

[l] Gas chromatography/Hall electrolytic conductivity detector.

[m] Colorimetry.

[n] Nuclear magnetic resonance.

3. Most metals and metal-containing compounds can be analyzed by atomic absorption spectroscopy (Section 4) or inductively coupled argon plasma (Section 7). When the metals are in solid matrices such as hair and tissue, they can be determined by neutron activation analysis (Section 5), and proton induced x-ray spectroscopy (Section 8) without further sample preparation. Some specific metal matrix combinations may be best analyzed by electrochemical methods (Section 6) or colorimetric methods (Section 9). Other techniques applicable to the analysis of metals and organometallic compounds can be found in Sections 13 and 14, however, they do not appear to be as wide spread and convenient as the ones mentioned above.

SECTION 4

ATOMIC ABSORPTION SPECTROPHOTOMETRY

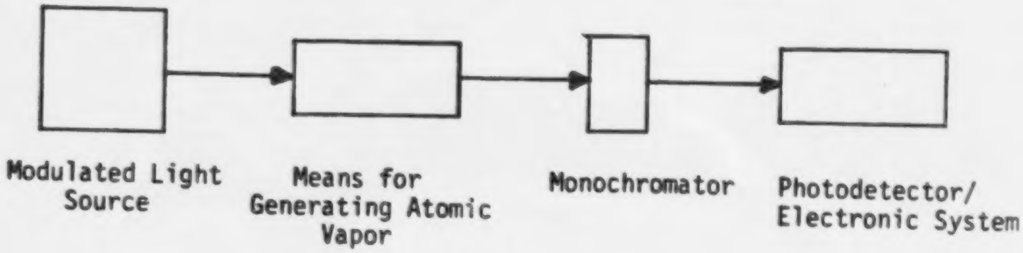
INSTRUMENTATION

Principle of the Technique

During the operation of an atomic absorption spectrophotometer, a spectrum of emission lines is radiated from the light source containing a cathode of the metal of interest and directed through the atomic vapor generated from the sample in a flame or other device. The metal of interest present in the ground atomic state absorbs the element-specific radiation from the light source. The monochromator isolates the resonance line selected for measurement and the detector/electronic system processes the signal output and records/displays the amount of analyte in the sample. A brief description of the instrumentation required for this analysis is given below.

Essentially, an atomic absorption spectrophotometer consists of four basic components:

- A modulated light source which generates an emission spectrum of the element of interest,
- A mechanism by which a portion of the element of interest in the sample is converted to the ground atomic state,
- A wavelength selector to isolate the resonance and nonabsorbable lines in the spectrum, and
- A photodetector/electronics system


Modulated Light Source
Means for Generating Atomic Vapor
Monochromator
Photodetector/ Electronic System

Light Source--

By far the most common atomic absorption light source is the hollow cathode lamp (HCL). HCLs consist of a cathode, usually in the shape of a cylinder, closed at one end and an anode of a straight piece of metal wire. The tube is sealed under a few millimeters pressure of a neon or argon filler gas. The face of the lamp is made of a material, usually quartz, which is transparent to the analytical wavelength.

With the passage of a current through the HCL, metal atoms are sputtered from the cathode surface and, on collision with the filler gas, are placed in an excited state and emit the radiation spectrum characteristic of that metal. Typically, the emission lines possess a half-width of 0.002 nm.

Although there is usually no particular reason for choosing between neon or argon as the filler gas, there are certain cases where one or the other must be used. Analytical performance during lead, iron, and nickel determinations is far better using neon rather than argon. Because of a strong neon emission line near the primary lithium and arsenic lines, the determination of these elements is better performed with an argon-filled lamp.

Over the past few years, advances in optimizing HCL performance has resulted in design features which give longer lamp life, greater spectral purity, and increased emission stability and intensity. These changes have resulted in signal output with less noise and a more sensitive working curve covering larger concentration ranges.

In an attempt to realize some economic and practical benefit, investigators have designed and tested several different multi- element lamps. Decreased warming times (single beam instruments), lower costs, and smaller storage areas are possible advantages of combining several elements into one cathode. The cathodes of multi-element lamps have been prepared from different types of materials: alloys, intermetallic compounds, and mixtures of powders sintered together. When three or more elements are combined in the cathode of a lamp, a decrease in brightness compared to that of the corresponding single element lamp has been observed. Multi-element lamps have been prepared containing up to six different metals (e.g., Ni-Co-Fe-Cu-Cr-Mn). The most serious problem with these lamps is due to the spectral interferences of the cathode components (e.g., a copper emission line only 0.25 nm distant from two iron lines). Although some of these interferences may be removed by narrowing the bandpass of the monochromator, these corrections are usually made with some loss in energy throughput.

Mechanism for Producing an Atomic Vapor--

Flame--During the early days of atomic absorption development, element vaporization was carried out by aspiration of the sample solution into a flame sufficiently hot to generate the desired atomization. The flame had to produce molecular dissociation with a minimum of vaporization. Typical flame mixtures and their temperatures are given in Table 4-1.

The position of the light beam in the flame and the fuel/oxidant ratio of the flame influence the analytical characteristics of any determination. Atomic absorption instruments allow adjustment of these parameters to permit operation at optimum conditions.

Two basic types of burners have been used in atomic absorption work: the total consumption or diffusion type and the premix type. The former burner is used in flame photometry but possesses significant disadvantages for atomic absorption work. The premix burner type involves the aspiration of the sample solution into a chamber containing the flame fuel and oxidant which mix and pass through the burner slot to the flame.

In the total consumption burner, the total sample passes to the flame, but only the smallest droplets are dried and burned. In the premix type, only the smallest droplets travel to the flame; the largest droplets collect on the walls and pass to the drain. Both types of burners are only 5% efficient (the proportion of sample which burns in the flame). Most atomic absorption instrumentation includes the premix type of burner assembly.

Main advantages of the premix burner type include:

- better linearity of working curves;
- low contribution of flame to analytical noise;
- fewer interferences; and
- potential to determine more elements.

Main advantages of the total consumption burner type include:

- faster response time; and
- lower memory effects.

TABLE 4-1. TYPICAL FLAMES FOR ATOMIC ABSORPTION

Flame Mixture	Flame Temperature, °C
Air - H_2	2050
Air - C_2H_2*	2350
O_2 - H_2	2500
N_2O - H_2	2600
N_2O - C_2H_2*	2955
O_2 - C_2H_2	3100

*Most commonly used.

A variety of burner heads are available. Specific burner heads should be used for specific flames. Within each "flame" category, burner heads with different lengths, widths, and number of slots are available for specific applications.

Flameless--Flameless atomic absorption procedures usually call for a graphite furnace (or carbon rod atomizer) for sample atomization. A relatively small volume of sample (10-25 μL) is deposited into the graphite tube or rod, evaporated to dryness, charred, and atomized. The temperature is controlled electrically and, on current instrumentation, the heating cycle for a particular determination can be programmed to temperatures of 3000°C at a controlled rate over as many as seven stages. The analytical principle is basically the same as flame atomic absorption except the furnace takes the place of the flame in atomizing the sample. The samples are prepared for analysis by the same procedures used for flame measurements. Detection limits of all elements are typically orders of magnitude lower than obtained by the comparable flame method.

The other flameless technique of note is the cold vapor method for mercury determinations. All forms of mercury are oxidized with potassium permanganate to the +2 valence state. The mercuric ions are then reduced by stannous chloride to the elemental form. The mercury atoms absorb the 253.7 nm resonance line as it passes through a gas cell positioned in the light path of a mercury hollow cathode lamp.

Other Vaporization Methods-- Hydride methods are commonly used in the determination of arsenic and selenium. In fact, analytical sensitivity is better than that obtained from procedures using flame techniques. The cold vapor mercury assay is another example of the generation of an atomic vapor by chemical means. Determination of mercury by this method is preferred to other atomic absorption techniques.

Wavelength Selector (Monochromator)--

The choice of a monochromator for an atomic absorption spectrophotometer is based on consideration of two parameters: energy and resolution. Energy is the amount of light which passes through the monochromator, and resolution is the minimum wavelength by which two lines can be distinguished. A higher energy and a lower resolution are desirable but, in practice, one is usually achieved at the expense of the other. For atomic absorption work, the two most widely used monochromators are the Littrow and Ebert-Fastie type. Their relative performance has been evaluated and indicates that there is little difference between the two methods.

The ability of atomic absorption to distinguish between elements depends on the emission line width of the HCL (0.002 nm) and the absorption line width of the element in the flame (0.004 nm). The commonly available monochromators are not capable of achieving such resolution. Thus, the monochromator can influence the quality of analytical performance, but it is not responsible for the specificity for which atomic absorption is noted.

The major function of the monochromator is to separate the resonance line of interest from the nonabsorbable lines emitted by the light source. The determination of elements (Ni, Fe, Co) which produce complex emission spectra require a resolution of 0.2 nm. In such cases, better resolution (e.g., 0.05 nm) will not improve analytical performance but, instead, will reduce energy throughput with concomitant loss in precision and poorer detection limits. A list of suggested resolution values is given in Table 4-2.

If the monochromator does not provide the necessary resolution, nonabsorbable lines will pass through to the detector. Analytical specificity will not be compromised, but the working curve will flatten prematurely with loss of sensitivity.

On the other hand, the emission spectrum of certain elements (e.g., zinc, arsenic, selenium, calcium, lead, and the alkali metals) are not complex and do not require the resolution needed for the elements discussed above.

The reciprocal linear dispersion (RLD) of a monochromator expresses its resolving power as a function of slit width. For example, if a monochromator had a RLD of 1.0 nm/mm, a change of 1.0 mm in slit width will produce a difference of 1.0 nm in resolution. In general, the lower the RLD the more sensitive the resolution is to slit width and the better suited it is for atomic absorption work.

TABLE 4-2. ATOMIC ABSORPTION DETECTION LIMITS

Element	Analytical Wavelength, nm	Flame Detection Limit, μg/L		Furnace Detection Limit,[b] μg/L	Suggested Resolution,[a] nm
		50% CV (1968)[a]	IL (1982)[b]		
Ag	328.1	5	2	0.001	0.7
Al	309.3	100	20	0.01	0.2
As	193.7	100	100	0.08	0.7
Au	242.8	20	10	0.01	0.7
B	249.7	6000	1000	-	0.7
Ba	553.5	50	20	0.04	0.4
Be	234.9	2	1	0.003	2.0
Bi	223.1	50	40	0.01	0.2
Ca	422.7	2	1	0.01	1.3
Cd	228.8	1	1	0.0002	0.7
Co	240.7	5	5	0.008	0.2
Cr	357.9	5	3	0.004	0.2
Cs	852.1	50	20	-	4.0
Cu	324.7	5	2	0.005	0.7
Dy	421.2	200	60	-	0.2
Er	400.8	100	40	0.3	0.2
Eu	459.4	80	-	-	0.4
Fe	248.3	5	5	0.01	0.2
Ga	287.4	70	50	10	0.7
Gd	368.4	4000	2000	8	0.2
Ge	265.1	1000	50	0.1	0.2

(continued)

TABLE 4-2. (continued)

Element	Analytical Wavelength, nm	Flame Detection Limit, μg/L		Furnace Detection Limit,[b] μg/L	Suggested Resolution,[a] nm
		50% CV (1968)[a]	IL (1982)[b]		
Hf	307.3	8000	2000	-	0.2
Hg	253.7	500	200	0.2	2.0
Ho	410.4	100	60	0.7	0.2
In	303.9	50	30	0.02	0.7
Ir	264.0; 208.8	2000	500	0.5	0.2
K	766.5	5	1	0.004	1.3
La	392.8; 550.1	2000	2000	0.5	0.4
Li	670.8	5	2	0.01	4.0
Lu	331.2	3000	-	-	-
Mg	285.2	0.3	0.2	0.0002	2.0
Mn	279.5	2	3	0.0005	0.7
Mo	313.3	30	10	0.03	0.2
Na	589.0	2	0.4	0.004	0.4
Nb	504.9; 334.9	3000	2000	-	0.2
Nd	463.4; 492.5	2000	700	-	0.2
Ni	232.0	5	8	0.05	0.2
Os	290.9	-	90	2	-
Pb	283.3; 217.0	30	20	0.007	0.7
Pd	247.6	20	20	0.05	0.2
Pr	495.1	1000	2000	-	0.7
Pt	265.9	100	50	0.2	0.7

(continued)

TABLE 4-2. (continued)

Element	Analytical Wavelength, nm	Flame Detection Limit, μg/L		Furnace Detection Limit,[b] μg/L	Suggested Resolution,[a] nm
		50% CV (1968)[a]	IL (1982)[b]		
Rb	780.0	5	2	0.1	0.2
Re	346.1	1000	800	10	0.2
Rh	343.5	30	2	0.1	0.2
Ru	349.9	300	400	-	0.2
Sb	217.6	100	60	0.08	0.2
Sc	391.2	100	20	-	0.7
Se	196.0	100	100	0.05	2.0
Si	251.6	100	60	0.6	0.2
Sm	429.7	2000	500	-	0.2
Sn	224.6; 235.5	20	80	0.03	0.7
Sr	460.7	10	6	0.01	1.3
Ta	271.5	5000	800	-	0.2
Tb	432.7	2000	1000	-	0.4
Te	214.3	100	70	0.03	0.7
Ti	364.3	100	50	0.3	0.2
Tl	276.8	25	30	0.01	2.0
Tm	410.6	150	-	-	0.2
U	351.4; 358.5	30000	7000	30	0.2
V	318.5	20	20	0.1	0.7
W	400.8; 255.1	3000	500	-	0.2

(continued)

TABLE 4-2. (continued)

Element	Analytical Wavelength, nm	Flame Detection Limit, µg/L		Furnace Detection Limit,[b] µg/L	Suggested Resolution,[a] nm
		50% CV (1968)[a]	IL (1982)[b]		
Y	407.7; 410.2	300	200	10	0.2
Yb	398.8	40	-	0.01	0.2
Zn	213.9	2	0.6	0.001	2.0
Zr	360.1	5000	2000	-	0.2

[a] H. L. Kahn, Principles and Practice of Atomic Absorption from Advances in Chemistry Series, No. 173, Trace Inorganics in Water, American Chemical Society, 1968.

[b] Instrumentation Laboratory, Inc., Guide to Analytical Values for IL Spectrometers, 1982.

Photodetector/Electronic System--

Two different types of photomultiplier tubes are used in atomic absorption work: Li-O-Ag cathode and Cs-Sb cathode. The former type is superior in the far UV (arsenic and selenium) and in the red (potassium) region; the latter is the more commonly used in the 220-580 nm range.

In atomic absorption spectrophotometry, concentration is proportional to the negative logarithm of transmittance over the working concentration range. Digital readouts are usually linear in transmittance or absorption and, on commercial atomic absorption instrumentation, can also be connected to a recorder or a printer.

Scale expansion can be employed when working at low concentration levels. However, amplifying the instrumental signal will also increase the fluctuation (noise) level of the output. By using readout damping (or increasing the time constant), the system becomes slower to respond to signal changes and, as a result, will minimize the noise level. This advantage is achieved at the expense of longer sampling times.

All modern atomic absorption spectrometers have microprocessor capability which, after calibration, will give results of analyzed samples directly in terms of concentration. This feature makes it unnecessary to relate absorption (or absorbance) readings to a standard curve and convert the experimental data to concentration values.

Most digital readout accessories are programmed to extend the working curve and to report the analytical measurement by averaging replicate determinations of the element of interest.

Chemicals Analyzed

Approximately 65 elements have been determined by atomic absorption spectrophotometry (see Table 4-2). Although some elements are better suited to this analytical technique than others, the method is very adequate for a wide variety of elemental types. Noticeably absent from the list of analytes are the inert gases, the halogens, and the nonmetallics including sulfur, oxygen, phosphorous, and nitrogen.

Methods of Quantitation

The key to converting raw atomic absorption data to analyte concentration in samples is to insure that potential sample interferences have been taken into account and that the reported values do not reflect a sample-related bias. Certain types of interferences, associated with the sample matrix, can so distort the analytical response that aqueous standards are not appropriate for quantitation. There are check procedures to determine whether calibration with aqueous standards is valid for a given sample type. The approach is based on the recovery of spikes, and the acceptability criterion is usually set at ±10-15% of theoretical.

A certain amount of nonspecific background can be compensated for by incorporating a deuterium arc into the system. Alternatively, the amount of background in a particular determination may be estimated by measuring the absorbance at a nearby nonabsorbing line. It may turn out that, even with deuterium background correction, an aqueous standard curve does not adequately describe the analyte concentration _vs_. absorbance relationship. One approach is to (tentatively) identify the cause of the problem and try to match (with respect to the samples) the matrix in which the standards are prepared. For example, if ionization of another element, presence of organics, high solids content, or a high anionic content is thought to be responsible, then those factors should be incorporated into the standard matrix. Alternatively, the causative factor can be eliminated or minimized in the samples themselves by instituting a chemical or physical removal treatment (e.g., heat, chelation, extraction, or combustion). The objective of the above procedures is to make sure that the concentration _vs_. absorbance correlation of samples and of standards is sufficiently comparable to permit quantitation.

When the sample matrix is either too difficult to match or when the appropriate sample pretreatment is not practical, the technique most commonly used is termed the "method of standard additions". Each sample is spiked at two or three levels and the increase in absorbance relative to the unspiked solution is noted. The data may be presented as shown in Figure 4-1. The regressed line is extrapolated to the (-X) axis and the concentration read as

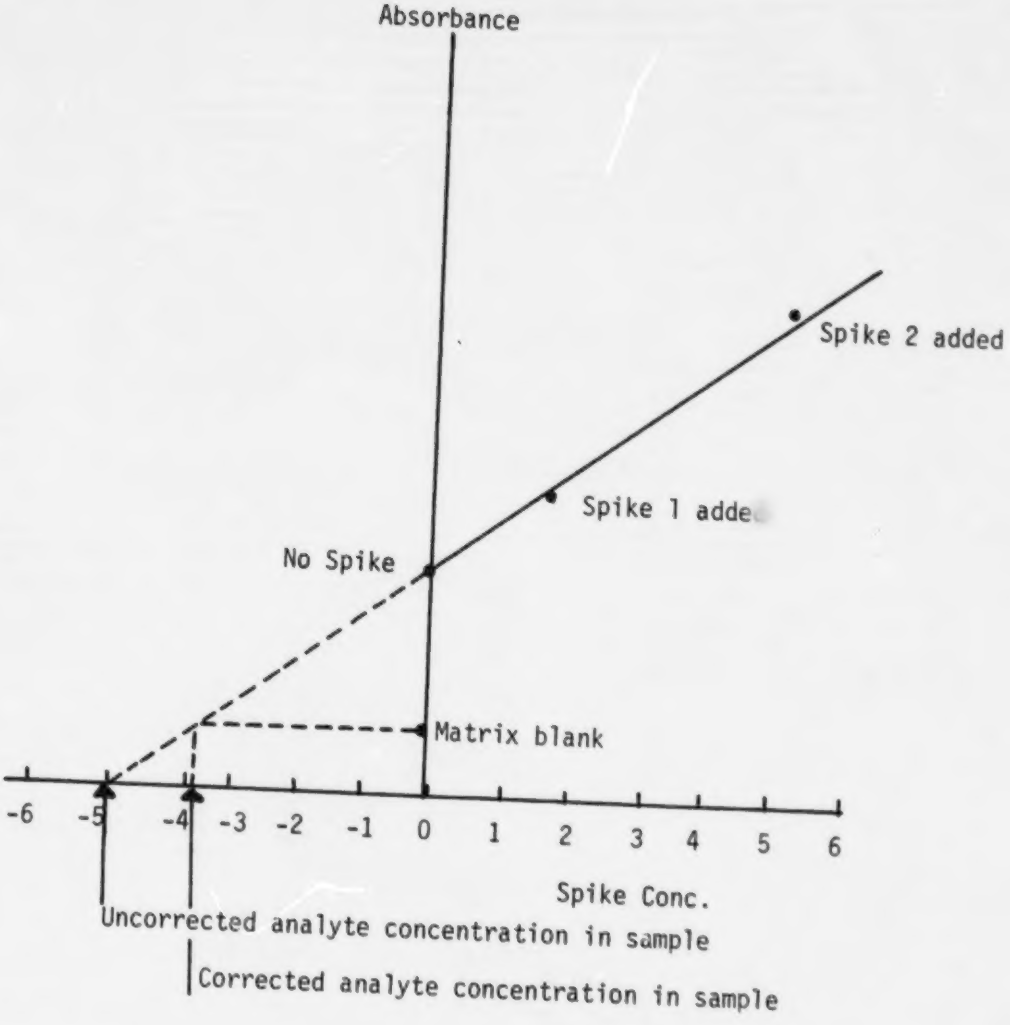


Figure 4-1. Method of standard additions.

the absolute value of the (-X) intercept. Care must be taken that the spiked solutions remain in the linear range of the working curve.

The spikes are normally added after sample workup and before introduction to the instrument. Calculations from this treatment will not take workup recovery into account. If the spikes are added before sample workup, the observed analyte concentration will be adjusted for recovery.

If a particular matrix material (e.g, blood) is scheduled for analysis, a pool of matrix material may be used as being representative of the sample matrix and spiked accordingly either before or after workup. See Figure 4-2 for sample calculations.

It should be noted that the method of standard additions is not a technique for eliminating all analytical bias. The effect of molecular absorption interferences are not negated by standard additions.

Limit of Detection

Since the terms sensitivity and detection limit are used frequently in the atomic absorption literature, a definition of the terms is given below. Sensitivity is a measure of the slope of the calibration curve and is expressed as the elemental concentration (ppm) which produces a 1% absorption (absorbance = 0.0044 units) in the flame. On the other hand, detection limit is defined as the minimum sample concentration which is possible to distinguish from zero or a blank response.

There are several different approaches in estimating detection limit. Many investigators report detection limits based on instrumental considerations or on theoretical grounds. A much more meaningful set of limiting concentration values is based on direct measurement observations. One popular definition of detection limits in atomic absorption analysis equates this parameter to the elemental concentration which gives a signal twice the peak-to-peak variability of the background. In other words, the concentration of an element at its detection limit can be determined with a coefficient of variation (CV) of 50%. Alternatively background variability can be determined from a low level standard solution containing no more than five times the detection limit eventually claimed. Detection limits calculated in this fashion are shown in Table 4-2 under 50% CV (1968).

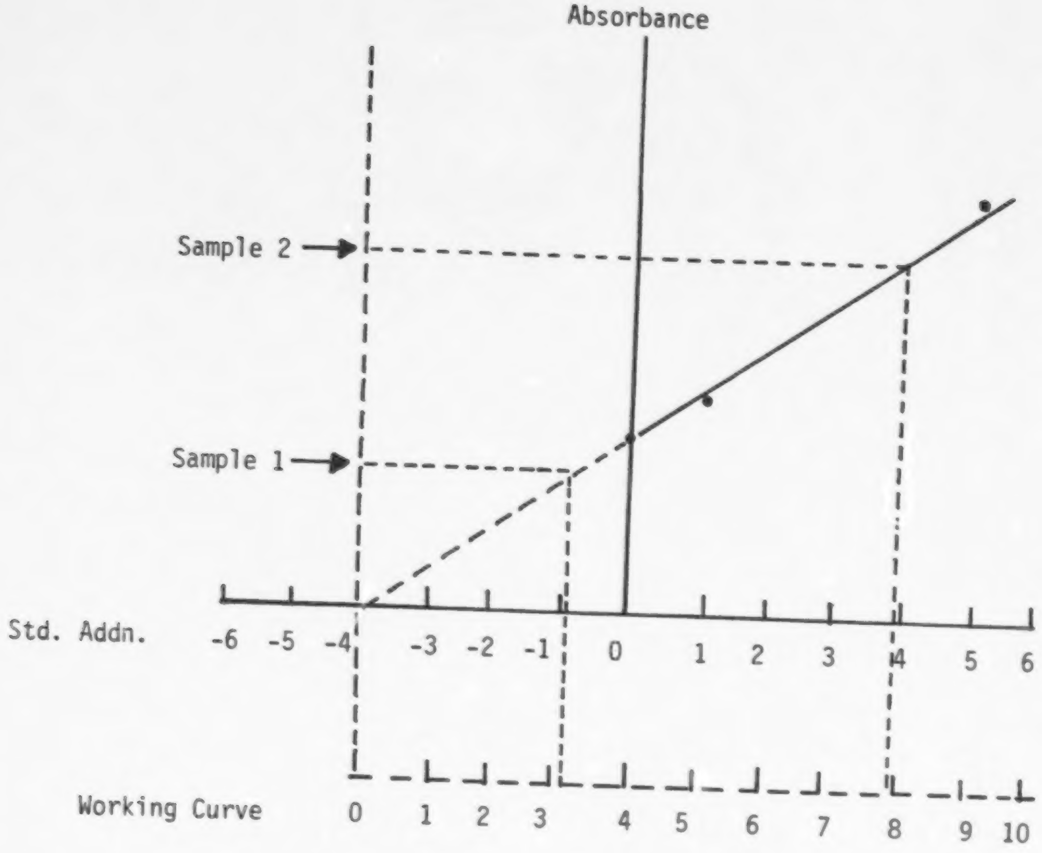


Figure 4-2. Standard additions working curve.

Instrument design and analytical conditions employed affect the detection limits of all analytes. The inherent characteristics of the optics and electronics of the analytical system will, in large measure, determine sensitivity and background variability. The detection limits will also be affected by the condition of the optical and electronic components and by factors such as the use of a light source if operating improperly.

Analytical conditions can also influence detection limit values. The use of nitrous oxide/acetylene flame instead of the cooler acetylene/air flame will result in low detection limits in many cases. With few exceptions, the use of electrothermal (furnace) atomization techniques yield detection limits which are orders of magnitude lower than those realized by flame methods. See Table 4-1 for the relative detection limits using common flame and flameless techniques in modern instrumentation.

Precision

The analytical precision of a set of atomic absorption measurements is normally expressed as percent relative standard deviation (%RSD). This parameter is determined by dividing the standard deviation of replicate determinations by the mean of the individual values times one hundred percent.

The precision determined for a replicate set of measurements is the result of the individual contribution of a number of factors. Precision values of 5-30% RSD are typical; the exact value for instrumental precision depends in part, on the variables discussed below.

Element Volatility--

The properties of the element under analysis can influence the overall method precision. For example, elements (e.g., As, Cd, Hg) with high vapor pressures possess a characteristic which may lead to (volatility) losses unless an appropriate amount of control is placed on sample workup. Losses may also occur during the furnace charging cycle unless suitable matrix modification procedures are implemented. The tendency for such losses to be variable aggravates any precision problem.

Interferences--

Interferences of any kind in the sample material usually affect analytical precision. Samples containing interferences due to ionization, molecular absorption, or chemical effects normally require a more extensive workup method. Moreover, the variable effect of the interference produces erratic reproducibility and poor precision. Thus, the determination of lead in water is associated with a lower %RSD than its determination in blood, urine, or soot.

Analyte Concentration--

Analytical precision will deteriorate as the analyte level approaches the detection limit. This is a logical consequence of the atomic absorption process at ultra trace levels. It is becoming common practice to set the detection limit at a concentration level which corresponds to a precision of 50% relative standard deviation (%RSD).

Contamination--

One factor contributing to overall precision which is often not considered is random contamination of glassware and other laboratory equipment. At times, it can overwhelm the contribution of other factors. The most insidious feature of this contamination is its randomness; it is very difficult to control without following a comprehensive cleaning protocol.

Accuracy

The analytical accuracy of a set of atomic absorption measurements is normally expressed as percent recovery of a spike or the percent bias from the analysis of a standard reference material (SRM). Percent recovery is determined by dividing the weight of spike found by the weight of added spike times 100 percent.

Percent recovery of an analyte spike is estimated by adding a known amount of the element of interest (spike) to the sample and repeating the preparation and analysis methodology used on the unspiked material. Typically, percent recovery data for elemental analysis by atomic absorption procedures ranges between 70-120%. This parameter is a function of several variables, the most important of which include nature of the sample preparation, element of analytical interest, interferences, mode of calibration, and concentration level relative to the estimated detection limit.

SAMPLE PREPARATION METHODS

Sample preparation methods have been placed in one of three categories.

(1) Complexation/Extraction Techniques,

(2) Ashing Techniques, and

(3) Sample Dilution Techniques

All literature citations which describe metal complexation and extraction as the last operation prior to analysis are included in that section. Chelation of sample elements with ammonium pyrrolidine dithiocarbamate (APDC) followed by methyl isobutyl ketone (MIBK) extraction comprises most of the literature is this area.

Ashing techniques are divided into wet, dry, and low temperature ashing operations. Another ashing procedure which is included in this category is the Delves or modified Delves cup method. As for the above mentioned method, literature citations are included in this category if the ashing step was the last operation prior to analysis, except arsine formation. For this summary, Delves cup ashing is not considered part of the analysis, but part of the sample workup.

Finally, all preparation methods which involve sample dilution with a solvent or reagent followed directly by analysis are included in the final category.

Since this section is based on mode of sample preparation, literature citations which did not indicate workup methodology were not included in this summary.

Complexation/Extraction Techniques

Principle of the Technique--

Complexing the element of analytical interest and extracting the reaction product with a water-immiscible organic solvent is a commonly used technique to remove chemical and matrix interferences and to concentrate the elements participating in the reaction. Ammonium pyrrolidine dithiocarbamate is the reagent normally used for this purpose since it readily forms stable complexes (chelates) which are extracted completely in a suitable solvent such as methyl isobutyl ketone (MIBK). Other complexing agents for specific applications include tri-n-octylamine, concentrated HCL/40% KI, dimethylglyoxime, and furildoxime.

Biological Matrices--

Ammonium Pyrrolidine Dithiocarbamate (APDC)--Blood must be hemolyzed (lysed) before APDC chelation can be performed successfully. Several different techniques for rupturing the erythrocyte cell wall have been employed including treatment with trichloroacetic acid (1,2), detergent (3), saponin (4,5), and Triton X-100 (6-9) and ashing (10-13). Plasma proteins have also been denatured and removed from the analytical matrix before reaction with APDC (1,14,15).

Similarly, urine samples have been pretreated in different ways prior to chelation: wet ash (11,16), wet ash with HNO_3/H_2SO_4 (13,17,18), adjust pH to 5.5 (19), adjust pH to 7.0 (7), wet ash with nitric acid followed by evaporation to dryness (15), wet ash with $HNO_3/HClO_4/H_2SO_4$ (20), treatment with trichloroacetic acid H_2SO_4 (14) and Triton X-100 (9).

Other Complexing Agent--Urine samples have been treated with a mixture of concentrated HCl and 40% KI to form toluene-soluble arsenic compounds (21). Alternately they have been subjected to a dry ash at 500°C followed by an aqua regia wet ash before treatment with stannous chloride and tri-n-octylamine in xylene (7). The results from a round robin study describe urine workups involving metal complexation with dimethylglyoxime or furildoxime (22). Serum has also been treated with dimethylglyoxime (23).

Blood and hair samples were wet ashed with a nitric acid/perchloric acid mixture before treatment with stannous chloride and tri-n-octylamine in xylene (7).

Blood and urine have been treated with mineral acids followed by chelation with sodium diethyldithiodicarbamate and extraction with MIBK (13,24-26).

Zinc in sera has been complexed with 8-hydroxyquinoline, cadmium in sera has been chelated with tetrahexylammonium iodide in MIBK (27).

Dithizone complexation and chloroform extraction has been used in the preparation of urine samples (13,28).

Chemicals Analyzed--

Ammonium Pyrrolidine Dithiodicarbamate (APDC)--APDC forms stable chelates with many elements under a wide range of reaction conditions. This characteristic explains, in part, the popularity of this reagent and its general acceptance as one of the most useful agents in preparing samples for metal analysis. The elements complexed by APDC and extracted with methyl isobutyl ketone (MIBK) include: Ni by graphite furnace (9,15,23); Cd by graphite furnace (1,9,13,18,20,29), by flame (12), and by tantalum ribbon (4); Pb (2,10,11,16,30) by flame (3,12) and by graphite furnace (5,7,8,19,31,32); and Cd, Pb, and Zn by flame (3,6).

Other Complexing Agents--Treatment of digested urine, blood, and hair samples with a stannous chloride:tri-n-octylamine mixture converted palladium residues to xylene-soluble products which were analyzed directly by graphite furance methods (7).

Urine samples were treated by wet or dry ash techniques before complexation of nickel with either dimethylglyoxime or furildoxime. The nickel chelate was extracted with MIBK and analyzed directly by graphite furnace methods (22,23).

Matrix interferences in urine have been removed by treatment with 40% KI and concentrated HCl. Arsenic bodies were converted to toluene-soluble products and, subsequently, back-extracted into 1% HNO_3 containing $Co(NO_3)_2$ (21).

Selective extraction of arsenic species by toluene extraction has been observed when substituting other mixtures for the KI-HCl reagent mentioned above (21).

Speciation has also been achieved by controlled arsine generation (21).

Lead in blood and cadmium in urine and blood were chelated with sodium diethyldithiocarbamate and extracted with MIBK (24-26).

Zinc in sera has been treated with 8-hydroxyquinoline and cadmium in sera has been chelated with tetrahexylammonium iodide in MIBK (27).

Method Interferences--

Ammonium Pyrrolidine Dithiocarbamate (APDC)--Deuterium background correction should be used to minimize nonspecific background interferences (1,4,31).

Other Complexing Agents--Deuterium background correction should be used to minimize nonspecific background interferences (1,27).

An interference study has been carried out on the analysis of serum samples for zinc and cadmium levels (27).

Concentration Factor - LOD--

The detection limit (signal:noise ratio = 2) of measuring lead levels in blood and urine was reported as 21 ppb and 4.7 ppb, respectively (7). The same limits for cadmium determinations were reported as 0.13 and 0.14 ppb, respectively (9). A round robin study for the analysis of urine for nickel content ranged between 0.5 to 10.0 ppb (22). The detection limit of palladium determinations in blood and urine was estimated at 0.4 ppb and 0.007 ppb, respectively.

Sensitivity data have been reported for lead determinations in blood plasma: 1.0 ppb (31) and 2 ppb (32). This parameter is defined as the concentration corresponding to 1% absorption.

Recovery Data - Quantitation Method--

Ammonium Pyrrolidine Dithiocarbamate (APDC)--Cadmium and lead were chelated and extracted quantitatively with MIBK by subjecting aqueous standard solutions to treatment with Triton X-100 and APDC. The recovery of zinc under the same conditions was 32%. Based on these findings, instrument calibration was carried out with aqueous standards and, in the case of zinc, corrected for recovery (6). A nickel spike added to urine was completely recovered from a HNO_3 wet ashing followed by APDC, chelation and extraction with MIBK (15).

Aqueous standards were submitted to sample workup to provide the calibration data for blood lead assays (2).

Determinations of lead in blood were based on standard aqueous solutions (5) or spiked plasma (32); lead in urine calibrations were carried out by the method of standard additions (19). Recovery of lead from plasma was reported as 85-91% (32). Determinations of cadmium in urine and blood were based on a series of spiked matrix composites (9). The cadmium recovery from urine and blood was 101.8% and 98.5%, respectively (9).

Other Complexing Agents--The results of a round robin of nickel measurements in urine samples showed data based on both aqueous standards and standard additions calibration (22). Recovery of nickel spikes added to urine was reported by participating laboratories and ranged between 88-110%.

Precision and Accuracy--

Ammonium Pyrrolidine Dithiocarbamate (APDC)--The precision of nickel determinations by furnace methods was estimated at 4.6-5.7% coefficient of variation (CV) for urine and 9.7% CV for plasma samples (15). At nickel concentrations below 20 ppb, the precision for the urine analysis falls to 11.6% CV. Other investigators reported CVs of 11.9% and 12.2% for the blood and urine assays, respectively. The nickel determinations in plasma samples which require APDC chelation before pretreatment (e.g., trichloroacetic acid or acid digestion) possessed a negative bias of 20% (15).

Within-run precision for lead measurements in plasma was reported as 1.7-5.0% CV (32). Precision for lead determinations in urine was shown to be 8.2% CV. Accuracy of urine and blood analyses was assessed by split sample analysis. Interlaboratory agreement was within the estimated range of variability (16).

A thorough evaluation of the wet/dry ash chelation analysis of urine and serum for nickel content has been carried out (23).

Precision for cadmium measurements in urine and blood was 8.5% CV and 5.9% CV, respectively (9).

Other Complexing Agents--The interlaboratory round robin precision for nickel measurements in urine varied by sample from 5-49% (22).

Simplicity, Cost, and Sample Throughput--

Compared to other comparable atomic absorption preparation methods, the chelation/extraction procedures involve more sample manipulation and are more time-consuming. Assuming the same type of analysis (i.e., flame vs. furance), there is no offsetting increase in sample throughput as a result of the chelation/extraction step; therefore, the cost of analysis would probably be higher than the cost based on other workup methods (e.g., acid digestion). It should be pointed out that the more expensive chelation/extraction procedures can lead to a significant reduction of matrix and chemical interferences during analysis with a concomitant improvement in precision and accuracy and a potentially lower detection limits.

The chelation/extraction methods have been evaluated against acid digestion and found superior in some cases (14). APDC has been shown to be a more useful chelating agent than dimethylglyoxime and furildioxime (14).

Approximate Cost: $10-30/element/sample

Approximate Sample Throughput (assuming typical sample preparation facilities):

15-20 samples prepared/day
60-80 determinations/day* by furnace
200-300 determinations/day* by flame
* Standard addition analysis on each sample would halve analytical throughput.

Recommended Method/Analyte Correlation Parameters--

Ammonium pyrrolidine dithiocarbamate (APDC) in methylisobutylketone (MIBK) is widely used in cases where the amount of element is not sufficient to determine directly or when the sample contains considerable dissolved solids. APDC chelates with many metals and the MIBK removes the complexes from the sample mixture. Metals which are particularly amenable to this treatment include zinc, cadmium, iron, copper, silver, lead, and chromium (hexavalent, not trivalent). APDC does not react with aluminum, beryllium, barium, and strontium. Certain metal chelates; e.g., Mn-APDC are not stable and will disassociate on standing.

Ashing Techniques

Principle of the Technique--

Ashing methods have been divided into four categories: (1) wet, (2) dry, (3) low temperature, and (4) Delves cup.

Wet ash methods normally involve the use of mineral acid(s) and heat, nitric acid alone or in mixture with other acids usually comprise the ashing mixture. The acids for a particular application are based on two considerations: the conversion of the analyte to a soluble form and the destruction of the organic matrix.

Dry ashing is defined as any procedure which involves a heating period at 400°-600°C.

Low temperature ashing includes techniques such as heating with an infrared lamp and with an oxygen plasma generated from a rf source.

The Delves cup method is an ashing procedure in which the sample is placed in a cup or microboat, usually mixed with a reagent, dried and ashed on a hot plate, and placed directly into an air/acetylene flame.

Biological Matrices--

Wet Ash Methods--Hair samples have been prepared for analysis by treatment with a mixture of HNO_3 and $HClO_4$ (7,33-41). Hair has also been subjected to the action of HNO_3/H_2O_2 (4), acid digestion (42-46), and wet oxidation (47-48).

A mixture of $HNO_3/HClO_4/H_2SO_4$ has been used as a digestion mixture for urine (14,44,49,50) and plasma (14). Urine has been prepared by acid digestion (51-53).

Blood has been treated with HNO_3/H_2O_2 (19), $HNO_3/HClO_3$ (39,54), and acid (44,55-57).

Nails have been prepared for analysis by wet ashing or digestion with HNO_3/H_2O_2; and skin samples, by treatment with 6N HCl (58) or by wet ashing (59). Fat samples have been prepared for analysis by wet ash techniques (9).

Dry Ash Methods--After washing with sodium lauryl sulfate and rinsing with water and acetone, hair samples have been dry ashed at 450°C-500°C in a silica crucible (60) or under other conditions (52,61-63). Skin samples have been defatted by extraction with petroleum ether/ether and then dry ashed at 550°C for 16 hours in a platinum crucible. After a short drying period, urine, serum, or blood samples have been charred at 700°C for 2 minutes in a microboat (64). Urine samples have subjected to dry ashing at 650°C in the presence of $Mg(NO_3)_2$ (65). A screening method for lead in blood involves dry ashing (66).

Low Temperature Methods--A low temperature asher has been used to combust the organic matrix in urine (67,68), serum (69,70), and hair (68,71). An infrared lamp was used for the same purpose with blood samples (72). Hair samples have been combusted in oxygen (73).

Delves Method--The Delves cup method (flame) or a modification thereof has been applied to blood (49,74-85), urine (75,77), and hair (80,86) samples. Pretreatment methods are implemented to either homogenize or stabilize the sample material using deionized water (75), shaking (76), aqua regia (77), 0.1N HCl (75), and Triton X-100 (87).

A modified technique using microboats for sample drying, charring, and eventual atomization in a graphite furnace has been reported for urine and blood (87).

Chemical Analyzed--

Wet Ash Methods--Cadmium has been determined in biological matrices after wet ashing (55,88) by flame (9,37,38,44,49,53,56,75), graphite furnace (9,19), and tantalum ribbon (4). Lead has been measured in the same sample materials (33,34,47,89) by flame (7,37,44,50,53,75), graphite furnace (44), and tantalum boat (56).

Other elements determined after wet ashing include magnesium and calcium (40,55,58) by flame (37,54,90), zinc (36,40,42,46,47,51,55), by flame (37,44,59, 75,90), arsenic by flame (39,44), nickel by graphite furnace (14) and flame (37), and chromium (55) by graphite furnace (45,59).

Dry Ash Methods--Dry ash digests have produced solutions on which measurements of calcium and magnesium by flame (58), nickel by graphite furnace (64), zinc (61,62), cadmium by flame (60) and graphite furnace (52), arsenic (65), and lead (63) by graphite furnace (52) have been performed.

Low Temperature Methods--Low temperature ashing techniques have resulted in sample digests on which determinations of Cr by graphite furnace (51,53) and flame (68), Zn by flame (69,70), and lead by graphite furnace (72) have been carried out. Arsenic by flame (73) and Cd, Pb, and Zn (73) have been determined after this workup technique.

Delves Method--Lead (49,74,75,78-87) and cadmium (75-77,87) are the only two elements which were reported to have been measured by this technique.

Method Interferences--

Wet Ash Methods--Lanthanum salts were added to wet ash residue to prevent phosphate interference in the determination of magnesium and calcium in hair and blood (54,90). Other investigators claimed no interference in the analysis of hair for zinc, cadmium, lead, nickel, magnesium, and calcium (37). A deuterium background corrector was used to minimize the effect of nonspecific interferences in the analysis of blood for cadmium (19,53); the corrector was not needed for lead measurements (52). Potential matrix interferences are eliminated by treating hair with boiling water before ashing (33).

Delves Method--A deuterium background corrector was used to minimize the effect of nonspecific interferences in the analysis of blood for cadmium (76).

Matrix modification was investigated in an attempt to minimize analytical interferences. The lead in urine analysis was carried out in the presence of ammonium nitrate, and the cadmium in urine analysis was performed with added ammonium fluoride. Ammonium acid phosphate was used in the cadmium in blood assay to minimize analyte loss (87).

Concentration Factor - LOD--

The detection limit for determination of cadmium has been reported in hair, 0.13 μg/g (9); fat, 0.015 μg/g, 0.015 μg/g (9); and blood, 0.04 μg/g/100 mL (49). Limit of detection concentrations have been estimated for skin analysis of chromium (1 ppb) and zinc (400 ppb) content (59). Lead and palladium measurements in hair were reported to possess detection limits of 2.67 ppm and 0.6 ppb, respectively (7).

Recovery Data - Quantitation Method--

Wet Ash Methods--Recovery of lead spikes from urine was reported to be 95-105% (37); chromium recovery was 92% (59); zinc recovery was 96.6-103.1% (43). The recovery of Cd from hair and fat digestions was 97.3% and 149%, respectively (9).

For the determination of cadmium in hair, fat, and nails, standards were prepared in the matrix of interest (4,9). Aqueous standards were used in the quantitation of zinc, cadmium, lead, nickel, magnesium, and calcium in hair

(37). Aqueous standards were also used in the determination of cadmium in blood (19).

Low Temperature Methods--Chromium measurements in hair were based on the instrument response of aqueous standards (71). The calculation of lead concentrations in blood was carried out by the method of standard additions (72). The recovery of Cd, Pb, and Zn from an oxygen combustion of hair was reported as greater than 85% (73).

Delves Method--A standard additions method has been used for instrument calibration (66). No recovery data was available.

Precision And Accuracy--

Wet Ash Methods--Precision of nickel determinations in plasma after a wet ash workup was 26.0% coefficient of variation (CV); the same parameter for urine analysis was 38.2% (14). Chromium measurements in skin samples were reported with precision of 4.7% CV (59). The precision estimate of Cd and Pb measurements in urine was approximately 5% CV (53). The accuracy associated with the determination of As in hair, blood, and urine was ±5% based on NBS reference material (39).

Dry Ash Methods--Precision of cadmium measurements in hair and nickel determinations in urine, serum, and blood have been reported as 12.9% CV and 3.3-10.0% CV, respectively (64,91). Precision of lead determinations in hair was 3.8-5.5% CV (63).

Low Temperature Methods--The analysis of hair samples for chromium content was checked against NBS SRM No. 1569 (3). The accuracy of a serum assay for zinc was checked in the same manner (69,70).

Delves Method--The lead in blood determination has a CV of 5.75%, indicating an overall reproducibility of 11.5% (95% confidence limits) (92). The national lead screening program has set a ±13 to ±15% error to be within the criteria of acceptable performance (92). The standard deviation of the lead in blood analysis over the range 0.19-0.99 μmol/L is ± 0.056 μmol/L (74). The cadmium in blood and urine assay is extremely accurate and very reproducible (77).

Simplicity, Cost, and Sample Throughput--

See discussion in Chelation/Extraction section.

Approximate Cost: $10-30/element/sample (based on wet ash method).

Approximate Sample throughput (assuming typical sample preparation facilities):

	Samples Prepared/Day
Wet Ash	25-30
Dry Ash	12-15
Oxygen Plasma	6-8

	Determinations/Day*
Furnace	60-80
Flame	200-300

*Standard addition analyses on each sample would halve analytical throughput.

Recommended Method/Analyte Correlation Parameters--

Ashing is probably the most common sample preparation procedure in use. Wet ashing is the most popular and involves heating the sample in HNO_3 either alone or in combination with another mineral acid (HCl, H_2SO_4, $HClO_4$). Acidic and oxidizing conditions are maintained to minimize the loss of metals due to volatility and adsorption on the surfaces of the container, to convert the analyte to a soluble and readily atomized form, and to destroy organic compounds in the sample matrix. Most metals can be subjected to wet ashing conditions. Special recommendations are given for certain elements: the use of aqua regia for palladium workups and the use of nitric acid instead of hydrochloric acid when preparing samples for silver measurements.

Dry ashing methods are less common but are potential alternatives to wet ashing. Low temperature ashing should be considered for applications involving the destruction of organic matrices containing volatile elements. Oxygen plasma techniques have been suggested for combusting matrices with large surface areas such as hair.

Sample Dilution Techniques

Principle of the Technique--

Sample dilution procedures involve the addition of a solvent or reagent without the use of heat or the formation of a product extracted with an organic solvent. These treatments have two major objectives: to hemolyze blood samples and/or to dilute the effect of sample interferences. In some cases, no sample preparation is described.

Biological Matrices--

Whole blood has been analyzed directly (44,47-49). However, it is usual practice to hemolyze the sample by the addition of deionized water (42,75,93-99), trichloroacetic acid (100), organic solvents (101), or Triton X-100 (19,93-97, 102-106) before analysis. Blood has been ashed in situ by introducing the sample to a graphite furnace, adding HNO_3 and initiating the normal furnace cycle (72). Chloroform has been added to whole blood followed by $HClO_4$. Centrifugation yields the aqueous phase ready for analysis (50). Soluene (a quaternary ammonium hydroxide in toluene) has been used as a solvent for blood analysis (89,107).

Urine has been analyzed directly (4,26,98,108) and by the addition of an equal volume of 0.1N HNO_3 (19,75).

After a thorough washing procedure, hair samples have been introduced directly to a graphite tube for analysis (109).

Fat and skin specimens have been dissolved in toluene prior to analysis (89).

Chemicals Analyzed--

Lead has been determined in biological matrices prepared for analysis by solvent dilution (89): flame (50) and graphite furnace (19,72,75,92-95,99,102-106).

The following elements have also been determined after similar sample workups: Zn (77), flame (56,76), graphite furnace (30); Cd, flame (4), graphite furnace (32) (14); Cr, Ag, and Ni, graphite furnace (81); Mg (26); and silver, graphite furnace (110).

Method Interferences--

An interference study has been carried out to determine the analytical parameters which are important in the determination of lead in blood diluted with Triton X-100. Physiological concentrations of iron, calcium, magnesium, chlorine, and phosphate do not affect the assay. However, the presence of both potassium and sodium depress the lead signal. No interference was noted for Triton X-100; there was no need to add this reagent to standards. Heparin, oxalate, EDTA, and citrate concentrations less than 8 g/L did not interfere with the analysis. Higher citrate levels depressed the lead response (93). To prevent phosphate interference serum was diluted with a 5% La solution (35,40) or Sr solutions (110) before magnesium determinations (35,40).

Deuterium background correction was used during lead in blood measurements to minimize nonspecific interferences (19,50,93-95,99,104). The same correction was used for cadmium determinations in urine (19). Atomizing urine below 2300°C causes luminescence which depresses the chromium signal (98).

Concentration Factor - LOD--

The detection limit of lead measurements in blood has been reported as 5 μg/100 g with a sensitivity of 10 μg/100 g (95). This detection limit was derived by multiplying the standard deviation of a low level lead standard by two. Chromium determinations in urine possess a detection limit of 0.1 μg/L.

Recovery Data - Quantitation Method--

The average recovery of lead spikes in blood after dilution with Triton X-100 was 98% (93). The addition of chloroform and perchloric acid to blood gives a ±2 μg lead/dL recovery of the target value between 10-150 μg/dL (50).

Calibration for lead measurements in blood diluted with Triton X-100 was carried out with aqueous standards (93) or spiked blood standards (104). The lead measurements in blood diluted with deionized water were carried out in triplicate and the system calibrated by aqueous standards, spiked blood, and standard additions (95). The calculation of lead and cadmium concentrations in blood and urine, respectively, was based on standards in dilute nitric acid (19). A standard additions calibration was performed for chromium determinations in blood (98).

Precision and Accuracy--

The precision of lead determinations in blood/Triton X-100 was estimated at 3.1% within run and 5.4% day-to-day (93,103). Whole blood analysis for lead content was carried out with a coefficient of variation (CV) of 4.5-6.7%; packed erythrocytes were also analyzed for lead with a CV of 2.7-3.3% (72). The mean difference between blood duplicates (addition of Triton X-100) is 0.98 μg/dL.

Simplicity, Cost, and Sample Throughput--

The methods described in this section are simpler and less time-consuming than the preparative procedures discussed previously. However, the presence of additional matrix material is likely to increase the potential for chemical interferences during analysis.

Approximate Cost: $5-20/element/sample

Approximate Sample throughput (assuming typical sample preparation facilities):

75-100 samples prepared/day

60-80 determinations/day* by furnace

200-300 determinations/day* by flame

*Standard addition analyses on each sample would halve analytical throughput.

Recommended Method/Analyte Correlation Parameters--

The suitability of direct analysis for a particular application is more a function of the matrix than of the element being determined. The more complex the matrix, the less attractive this technique becomes; e.g., plasma or serum would be better suited than whole blood. Relatively low or moderately volatile elements (Hg, As, Cd) are more likely to be adversely affected by sample interferences than the more refractory elements (Cr, V, Mo). Although this preparative technique may be suited for quick screening programs, the analytical accuracy, precision, and detection limit are compromised relative to that achieved using ashing or chelation methods.

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SECTION 5

NEUTRON ACTIVATION ANALYSIS

INSTRUMENTATION

Principle of the Technique

Analytical Method--

Neutron activation analysis involves the measurement of the radioactivity of a sample after exposure to neutrons for a given period of time (1,2). Most elements irradiated by thermal neutrons (<0.4 eV) are converted to radioactive species of the same atomic number though increased by one mass unit through an (n,γ) reaction. The gamma ray (γ) released immediately after neutron capture has an energy equal to the neutron binding energy plus the kinetic energy of the neutron. As an example

$$^{23}\text{Na} + n \rightarrow {}^{24}\text{Na} + \gamma$$

The ^{24}Na is radioactive and decays into ^{24}Mg with the release of a beta particle of maximum energy equal to 1.389 MeV and two gamma rays of 1.369 and 2.754 MeV, respectively. Sodium can thus be identified and quantified through measurement of the beta particle or the gamma rays.

Activation or irradiation is accomplished by placing the sample in an intense flux of neutrons for a length of time sufficient to produce measurable amounts of the radioactive species of interest. The amount of radioactivity A_o, expressed in disintegrations per second, existing at the end of the irradiation is given by

$$A_o = N\Phi\sigma FS$$

where

N = the number of target nuclei available

Φ = the neutron flux, in neutrons/cm^{2}sec

σ = the capture cross section, in barns (10^{-24}cm^2/nucleus)

F = the fractional abundance of the target nuclide

S = the saturation factor

Here

$$S = 1\text{-}10^{-0.693t_i/(2.3\ t_{1/2})}$$

where

t_i = the duration of the irradiation

$t_{1/2}$ = the half life, or time for one half of the radioactive material to decay.

During activation of finite duration, decay of some fraction of the radioactive atoms produced will occur. The saturation factor, S, represents the ratio of the amount of activity produced during the irradiation period to that produced in infinite time. At $t_i/t_{1/2}$ values of 1, 2, 3, 4, 5, 6...∞, S has corresponding values of 0.5, 0.75, 0.87, 0.94, 0.97, 0.98... 1.00. Therefore the radioactivity for a particular element will reach 98% of the saturation value for irradiation times equal to six half-lives. The saturation effect can be used to reduce interelement interferences of elements having widely different half lives; this is further described in the section on interference.

Neutron Sources--

Two types of neutrons are used for activation. These are thermal neutrons (<0.4 eV) and fast neutrons (>1 MeV) (3). Thermal neutrons are neutrons in kinetic energy equilibrium with the temperature of the surroundings.

A nuclear reactor can serve as a source of both thermal and fast neutrons. Thermal neutron fluxes available from a reactor range from 10^{11} to 10^{14} neutrons/cm^{2}sec. Portable sources of thermal neutrons are available. These depend on the reaction

$$^{9}\text{Be}\ (\alpha,n)^{12}\text{C}$$

and consist of an intimate mixture of an alpha emitter, such as ^{226}Ra, ^{210}Po, or ^{237}Pu with beryllium. These sources provide only about 10^7 thermal neutrons/cm^{2}sec.

The fission-spectrum flux from a nuclear reactor serves as a source of fast neutrons. Normally the samples are wrapped in a cadmium foil to prevent thermal neutrons from reaching the sample.

Detection--

Activation analysis generally involves measurement of gamma rays rather than beta particles. Three common detectors used to measure gamma rays are the thallium-activated sodium iodide, NaI(Tl), scintillation detector, the lithium-drifted germanium, Ge(Li) detector, and high purity Ge, solid-state detector (4).

The NaI(Tl) detector operates in the same manner as other scintillation detectors. When a gamma ray passes through the NaI(Tl) crystal, a pulse of visible or near-ultraviolet light is produced, which is detected by a photomultiplier tube. The magnitude of the light pulse is proportional to the energy dissipated by the gamma radiation in the scintillator, that is, the energy of the gamma ray. Thus the magnitude of the pulse, which is measured with appropriate analogue and digital electronics, may be used to identify the emitting radioisotope. The number of light pulses of a given magnitude (i.e., arising from a particular gamma ray energy) is counted and provides a quantitative measure of the amount of the radioisotope present. The NaI(Tl) detector has high gamma ray detection efficiency but relatively low resolution that is, inability to distinguish between gamma rays of similar energy. It is thus useful for samples where few gamma rays of different energies are being emitted.

The Ge(Li) detectors, on the other hand, have an order-of-magnitude greater resolution than NaI(Tl) detectors, but they are only 10% as efficient as NaI(Tl). The Ge(Li) is a semiconductor material. When a gamma ray passes through it, electron-hole pairs are created, and the charge produced is rapidly collected under the influence of a voltage applied across the semiconductor material. The number of electron-hole pairs produced is a function of the gamma ray energy, and thus, measurement of the magnitude of the resulting current pulse allows qualitative identification of the emitting radioisotope. The number of pulses of a given magnitude provides quantitative information (5).

One of the major problems encountered with this type of detection is coincident summing. When two gamma rays strike the detector at essentially the same time, the photo or current pulse produced will represent the sum of the energies of these two gamma rays. Detector and electronic response times have been optimized to reduce the magnitude of this problem. Another approach is to use electronic circuitry which detects and rejects distorted and thus overlapping pulses (6). Still another approach is to use two or more detectors. In this latter approach, pulses detected coincidently by the detectors are rejected and only pulses measured with the central detector are processed (3).

The result of detection, differentiation of pulse energies, and counting is an emission spectrum with counts (pulses) per minute as the ordinate and energy (MeV) as the abscissa. The signals from the various activation products, i.e., radioisotopes, show up as peaks on a moderately high background called the Compton continuum, which is the result of Compton scattering, that is, elastic scattering of gamma-ray photons by electrons. An application is to use NAA to determine select isotopes of particular elements. Gordon et al. (7) reported on the feasibility of introducing stable isotope tracers into infants for nutrition studies. Two species which would be measurable are ^{70}Zn which forms ^{71}Zn and ^{74}Se which forms ^{75}Se.

Instrumental Limitations--

The principal limiting factor in neutron activation analysis is the presence of interferences, that is, the gamma-ray signals from the various radioisotopes not being resolved. Tables listing potential interferences for each of the elements (and also examples of detection limits) are provided in a handbook by R.C. Koch (8). Example data follow:

Emission Sources	Possible Interferences
$^{75}As\ (n,\gamma)^{76}As$	$^{76}Se(n,p)^{76}As$ $^{79}Br(n,\alpha)^{76}As$
$^{75}As(n,p)^{75}Ge$	$^{74}Ge(n,\alpha)^{75}Ge$ $^{78}Se(n,\alpha)^{75}Ge$
$^{75}As(n,2n)^{74}As$	$^{74}Se(n,p)^{74}As$ $^{73}Ge(p,\alpha)^{74}As$

There are several ways to deal with this problem including pre- and post-irradiation chemical separation techniques, counting time optimization, and mathematical deconvolution techniques.

Sensitivity of NAA is influenced by three factors that diminish the significance of the measured signal (gamma ray): (1) background from Compton scatter and other sources, (2) interference from other nuclides emitting unresolved gamma energies, and (3) direct production of the same radionuclide by (n,p) or (n,α) reactions.

If the radioisotopes of interest emit only beta particles or if interferences from other radioisotopes are severe, chemical separation techniques may be employed. For example, following irradiation, the sample is dissolved and chemically equilibrated with a relatively large (but accurately known; ~10 mg) amount of the elements of interest to serve as carriers. The elements are then separated. The amount of carrier element recovered is quantified by some other technique so that results can be normalized to 100% recovery. This makes it possible to employ rapid, nonequilibrium separation techniques where recoveries as low as 50% are acceptable.

The techniques used may separate groups or individual elements (9). Techniques used include precipitation, distillation, chromatography and solvent extraction (3). Heydorn has, for example, determined arsenic in hair by irradiation, acid dissolution and then precipitation of the arsenic with thioacetamide (10). Following irradiation, fusion with sodium peroxide, and dissolution in sulfuric acid/hydrogen peroxide solution, osmium may be distilled with a recovery of 99.8%. Matrix activity due to sodium and potassium may be removed by eluting the irradiated dissolved sample through a bed of hydrated antimony peroxide (HAP) and potassium tungstate (3). Dang et al. (11) combined lyophilization, digestion, distillation and precipitation to separate arsenic from human tissue and milk. Cornelis et al. (12,13) also performed preirradiation separation chemistry to determine vanadium. Liver and serum samples were lyophilized and dry ashed prior to irradiation. Immediately after irradiation, the ash was dissolved in 3N HCl and vanadium extracted as the cupferronate in chloroform. The authors reported performing the extraction in 4.5 minutes. As noted, sodium and chloride are present at high levels in many biological samples and cause interference. Woittiez and Das (14) reported on the removal of the halogens by evaporation as their hydrides following acid digestion and then removal of sodium by eluting the mineralized sample through a column of hydrated antimony pentoxide (HAP). Biso et al. (15) reported a complex procedure for determining mercury in hair. Following irradiation for eight hours at 2.1×10^{13} n/cm^2 s and a decay time of eight hours, the hair was digested in acid with a mercury carrier added. Elemental mercury was then formed with hydroxylamine and ammonium hydroxide, separated from the acid digest, redissolved in acid and then precipitated again as the thiocyanate.

A short half-life for the irradiation product often requires that pre-irradiation separation chemistry be performed in order to maintain adequate sensitivity. Sabbioni et al. (16) performed acid bomb digestion of different fractions of serum proteins and then separated potential interferences, including Na, Cl, and Br, from the remaining metals before irradiating for three minutes and counting for three minutes to determine vanadium.

Numerous schemes have been developed for separation of groups with the majority employing ion exchange and solvent extraction (3). An example of a group separation scheme is given in Figure 5-1. Chemical separations are labor intensive, especially when large numbers of samples are to be analyzed. They may be necessary however when high levels of certain elements interfere with and prevent the determination of other, low-level elements.

A very common way to deal with interferences is to take advantage of the differences in the saturation factors, i.e., half-lives of the various elements. The approach is first to irradiate the sample for a short period of time so as to "saturate" the elements with short half-lives. Measurement is then performed, and the sample irradiated again, but for a longer period of time in order to "saturate" the elements with long half-lives. Following this irradiation, the activated sample is simply stored for a period of time until the emissions from elements with short half-lives have decayed to the point where they do not interfere with counting emissions from the elements with the longer half-lives. Example irradiation/decay times are given in Table 5-1. A combination of time delay and chemical separation was used to determine 18 elements in blood serum (Nakahara et al., 18). The elements requiring chemical separation (As, Cu, Mn, Zn) have relatively short product half-lives and therefore will suffer interference from ^{24}Na and ^{82}Br if they are not separated from these species.

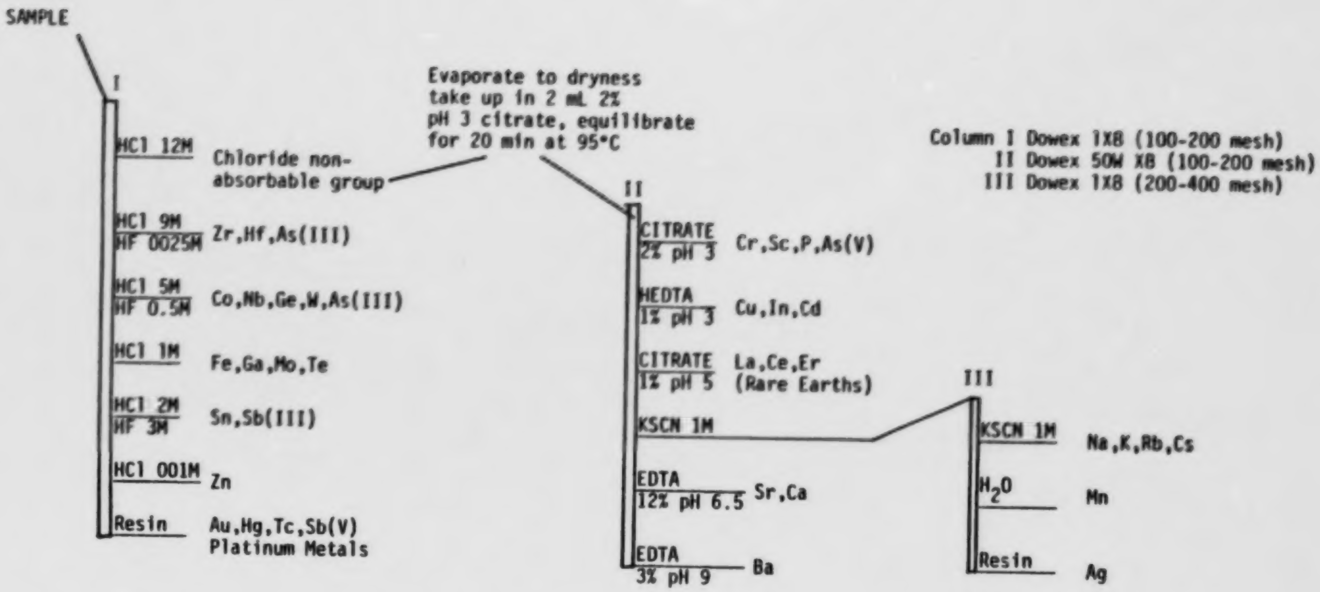
SAMPLE
I
HCl 12M
Chloride non-absorbable group
HCl 9M
HF 0025M
Zr,Hf,As(III)
HCl 5M
HF 0.5M
Co,Nb,Ge,W,As(III)
HCl 1M
Fe,Ga,Mo,Te
HCl 2M
HF 3M
Sn,Sb(III)
HCl 001M
Zn
Resin
Au,Hg,Tc,Sb(V)
Platinum Metals
Evaporate to dryness take up in 2 mL 2% pH 3 citrate, equilibrate for 20 min at 95°C
II
CITRATE
2% pH 3
Cr,Sc,P,As(V)
HEDTA
1% pH 3
Cu,In,Cd
CITRATE
1% pH 5
La,Ce,Er
(Rare Earths)
KSCN 1M
EDTA
12% pH 6.5
Sr,Ca
EDTA
3% pH 9
Ba
III
KSCN 1M
Na,K,Rb,Cs
H2O
Mn
Resin
Ag
Column I Dowex 1X8 (100-200 mesh)
II Dowex 50W X8 (100-200 mesh)
III Dowex 1X8 (200-400 mesh)

Figure 5-1. Group separation scheme of Jervis and Wong (17).

TABLE 5-1. IRRADIATION/DECAY TIMES FOR SELECTED ELEMENTS USING NEUTRON ACTIVATION

Elements	Irradiation/Decay Times
V, Mn, Al, Na, K	Hours
Sb, As	Days
Co, Cr	Months

Woittiez et al. (14) combined chemical separation and time delay to determine ten elements in human serum. One aliquot of serum was acid digested, the sodium removed through use of an ion exchange column, the sample irradiated for several minutes and then Mg, K and Cu determined. Another aliquot was irradiated, acid-digested and the arsenic and antimony converted to hydrides, the volatile hydrides collected on carbon and then counted. Finally, a lyophilized aliquot of the serum was irradiated for 12 hours and the elements Fe, Zn, Br, Rb, and Se counted during the weeks two through four following irradiation.

An irradiation time of five minutes was used to measure elements in human serum albumin having product half-lives less than 12 hours (Maenhaut et al., 19). For elements having irradiation products with longer half-lives, sample was irradiated for 32 hours and then counted during the periods of 3-5 days following irradiation and 20-30 days following irradiation. Several elements which suffer interference from sodium and chloride were separated from a mineralized sample aliquot using 8-hydroxyquinoline in chloroform; Mo was extracted at pH 1.1, Cu at pH 2, and Mn and Zn at pH 8.

Tomza et al. (20) used time delay to determine 24 elements in hair. For short-lived isotopes, irradiation lasted five minutes, the decay time three to five minutes, and the counting time 15 minutes. For long-lived isotopes, the irradiation period was seven hours and counting was done at 3, 10 and 30 days following irradiation. Hair was also analyzed using time delay by Turkstra et al. (21). Al, Cl, K, Mn, Na and V were determined after 15 minutes of irradiation and a 3.5 minute decay time. Cu, Br, Ce, Co, Cr, Fe, Hg, K, La, Na, Sb, Se, and Zn were determined after three days of irradiation and a 30-day decay time. Imahori (22) used three different delay periods (3 minutes, 48-72 hours, and 20-30 days) in their determination of 40 elements in hair. Seventeen elements were determined in hair by Dutkiewicz et al. (23) using time delay; irradiation time was 24 hours and counting time for the various elements were 1000 seconds after 4 to 8 hours, 2000 seconds after 1 to 2 days and 5 hours after 10 to 12 days. Behne and Jurgensen (24) analyzed human blood serum using time delay to control interferences. Irradiation was ten days at 5×10^{13} n/cm^2 s. Br and Na were counted after ten days and Ag, Co, Cr, Cs, Fe, Rb, Sb, Sc, Se, and Zn were counted after three months. The principal interferrent avoided with this delay was the bremstrahlung emission of ^{32}P. Molokhia et al. (25), in their analysis of skin, minimized interference from ^{24}Na, ^{42}K and bremstrahlung from ^{32}P by counting the elements of interest over a period of two to nine weeks following one week of irradiation.

The third approach to dealing with interferences is that of mathematical deconvolution. Techniques of deconvoluting overlapping signals have become more popular as computer capabilities have increased. These techniques are not useful however in those cases where the peak of interest is totally unresolved from a larger, interfering peak or multiple peak overlap is occurring. Usually interferences are dealt with through a combination of time resolution and mathematical deconvolution; chemical separations are performed only when absolutely necessary.

Chemicals Determined (Elements Determined)

Essentially all the elements of environmental interest (except beryllium) from nitrogen (Z=7) to the heavy elements can be determined using neutron activation analysis. Sensitivity is greater using fast rather than thermal neutrons for O, Si, P, Fe, Y, and Pb (4). Other elements which can be determined using fast neutrons include N, F, Al, Cr, Mn, Fe, Cu, Mo. and Nb. Pb cannot be determined using thermal neutrons.

Methods of Quantitation

The elements present are identified by the energies of their gamma-ray emissions. Thus, the accuracy of qualitative analysis is dependent upon precise measurement of the gamma-ray energy, which is generally 1-5%. The accuracy of the NaI(Tl) detector is about 10 keV which does not always allow an unambiguous identification. This is not the case with Ge(Li) detectors which have an accuracy of about 0.1 keV. Tabulations of gamma-ray energies are available for identification purposes (26,27).

For quantitative analysis, the areas of the gamma-ray peaks are determined using appropriate digital (hardware/software) techniques. These areas are then compared to those of standards for quantitation. One approach to quantitation is to use only one to three standards for which relative response factors have been determined for all elements of interest. Known quantities of these "internal standards" are irradiated along with the test samples, emissions from the standards and test samples are measured separately, and the relative response factors are used to calculate concentration. The difficulty with this approach is that experimental conditions must be maintained constant between the time of determining the relative response factors and the actual analysis. Another approach is to use standards for each of the elements to be determined.

Limits of Detection

Under conditions of high neutron flux (10^{12}-10^{14} n/cm^2s), maximum sample size (~1 g) and no interferences, neutron activation analysis provides detection limits which can only be matched by electrothermal or flameless atomic absorption spectrophotometry. The reported detection limits vary considerably, however. Table 5-2 lists some of these detection limits from various sources. The calculated detection limits reported by Lyon (29) appear reasonable in light of actual detection limits reported for specific studies. Included in the table of detection limits presented by Lyon is a list of biological and medical materials which have been analyzed for these elements; concentration ranges determined and references are presented. It must be strongly emphasized that these detection limits can be increased by one to three orders of magnitude (i.e., 10-1000 times) if significant interferences are present.

Precision

The precision achieved with NAA varies with the element to be determined, the concentration level of the element, the sample matrix and the extent of interferences. Typically, elements in the range of 10-100 µg/gm show precision in the range of 1-10%. Precision can be improved by counting the gamma-ray for extended periods of time.

Accuracy

The accuracy of NAA is difficult to establish at the low levels usually measured in biological/biomedical samples because of lack of accepted standards. Accuracy is sometimes tested by comparing a NAA result with that determined by another method. Versieck et al. (30) reported NAA and AAS results that agree within 4% for Cu and 6% for Zn at the ppm level in serum. The best indications

TABLE 5-2. DETECTION LIMITS FOR NEUTRON ACTIVATION ANALYSIS

Element	Detection Limit µg	Reference
As	0.005	28
As	0.002	29
As	0.0002	5
Ni	0.7	28
Ni	0.4	29
Ni	0.02	5
Hg	0.003	28
Hg	0.006	29
Hg	0.01	9

of method accuracy are provided by analysis of National Bureau of Standards, Standard Reference Materials. Ryan et al. (31) reported results which are shown in Table 5-3. Additional results of reference material analyses are presented in Tables 5-4 through 5-7. Versieck et al. (34) in their analysis of serum, used NBS Bovine Liver and Bowen's Kale to measure the accuracy of their system. The NBS Bovine Liver (SRM 1577) yielded 3.49 ± 0.05 μg/g whereas 3.2 μg/g was expected. The expected value for Bowen's Kale was 2.33 ± 0.47 μg/g and values of 2.08 and 2.23 μg/g were found. It should be noted that most of these elements are at generally high levels and accuracy at the parts-per-billion level is not yet fully established.

Simplicity, Cost, and Sample Throughput

The method of NAA is not simple because of the necessity of having a source of neutrons. With the exception of acquiring access to a neutron source, NAA can be relatively simple since most samples can be analyzed directly without any preparation. In the absence of interferences, data evaluation is also straightforward. Dealing with interferences by the method of time or half-life resolution is straightforward, but could result in an analysis time of weeks and possibly even months. Using chemical separation schemes to deal with interferences can make the overall procedure moderately to extremely complex.

In the absence of interferences, the cost for analysis ranges from $5-$10 per element per sample when quantifying approximately 20 elements per sample. This cost increases if extensive time-resolution is required and could increase by an order of magnitude if extensive chemical separations were required.

Sample throughput depends principally upon the levels of interferences and how they are dealt with. Turnaround time for a sample requiring two activation and counting steps is typically one to three weeks when the work is performed by a commercial laboratory.

SAMPLE PREPARATION METHODS

NAA is generally a nondestructive technique and sample preparation is usually minimal, reducing the chance of sample contamination considerably. Most samples are analyzed directly, some types are lyophilized, and in a few cases they are ashed or digested. For example, hair and nails were analyzed directly

TABLE 5-3. REPORTED ACCURACY FOR NAA (31)

		Concentration, μg/mg	
		Determined by NAA	NBS Certified
NBS Bovine Liver	Br	8.2 ± 0.1	8.5 ± 1
	Ca	125 ± 13	124 ± 6
	Cl	2680 ± 80	2610
NBS Orchard Leaves	Cu	12.1 ± 0.1	12 ± 1
	Na	81 ± 7	82 ± 6

TABLE 5-4. TRACE ELEMENT CONCENTRATION IN NBS BOVINE LIVER
Behne and Jurgensen (24)

Element	Replicate Number	Measured	Expected
Ag	18	0.068 ± .006	0.06
Br	16	9.0 ± 0.6	--
Co	21	0.24 ± 0.03	0.18
Cr	16	0.12 ± 0.07	--
Cs	21	0.013 ± 0.001	--
Fe	16	264. ± 29.	270 ± 20
Na	19	2550 ± 190.	2430 ± 130
Rb	18	19 ± 1.6	18.3 ± 1.0
Sb	18	0.007 ± 0.005	--
Sc	18	0.0011 ± 0.0003	--
Se	18	1.14 ± 0.11	1.1 ± 0.1
Zn	16	133 ± 7	130 ± 10

TABLE 5-5. TRACE ELEMENT CONCENTRATION IN NBS BOVINE LIVER
Lin (32)

Element	Measured	Certified or indicated values given by NBS
Co	0.19 ± 0.02	0.18
Cr	0.09 ± 0.014	0.088 ± 0.012
Fe	248 ± 16	268 ± 8
Rb	17.6 ± 1.2	18.3 ± 1.0
Sc	0.0011 ± 0.0002	0.001 ± 0.0001
Se	1.15 ± 0.11	1.2 ± 0.1
Zn	143 ± 8	130 ± 13

TABLE 5-6. TRACE ELEMENT CONCENTRATION IN NBS BOVINE LIVER AND BOWEN'S KALE

Molokhia et al. (33)

Element	Mean Concentration ± Standard Deviation, ppm	
	Kale	Bovine Liver
Sc	0.0112 ± 0.0011[a] (0.0082 ± 0.001)[b]	-
Cr	0.591 ± 0.105[a] (0.370) ± 0.089)[b]	-
Fe	123 ± 8[a] (118 ± 17[b]	271 ± 27[a] (270 ± 20)[b]
Co	0.086 ± 0.012[a] (0.0624 ± 0.011)[b]	0.247 ± 0.031[a] (0.254)[b]
Zn	32.04 ± 2.33[a] (33.70 ± 2.3)[b]	126 ± 5[a] (130 ± 10)[b]
Se	0.123 ± 0.01[a] (0.133 ± 0.021)[b]	1.03 ± 0.09[a] (1.10 ± 0.10)[b]
Rb	55.20 ± 1.8[a] (52.9 ± 4.4)[b]	17.72 ± 1.8[a] (18.30 ± 1.0)[b]
Cs	0.081 ± 0.021[a] (0.077 ± 0.006)[b]	0.0126[a] (0.019)[b]

[a] At least 10 determinations made.

[b] Certified or best values.

TABLE 5-7. TRACE ARSENIC CONCENTRATION IN NBS AND IAEA[a] REFERENCE MATERIALS
Dang et al. (11)

Reference Material	Measured (ppm)	Literature
NBS Bovine Liver	0.060 ± .006	0.056, 0.055, 0.059
IAEA Wheat Flour	0.020 ± .002	0.029, 0.022
IAEA Milk Powder	0.004 ± .001	0.005, 0.005
IAEA Animal Muscle	0.005 ± .001	0.007, 0.006

[a] International Atomic Energy Association (Vienna, Austria).

after washing in deionized water or some other cleaning agent, though the effects of the washing remain uncertain (35,36). Other substances which were analyzed directly were urine and serum (36). Skin has been analyzed directly after drying (37). Lyophilization or freeze drying is a common sample preparation technique. It has been used for food (36), placenta, maternal and fetal blood (38), and urine (39).

The samples are usually sealed in polyethylene ampules after preparation and submitted to irradiation in this container. When large quantities of biological material, for example, serum, are used, and the neutron flux is extremely high (10^{14} n/cm^2s), decomposition will occur, gas will be produced, and there is danger of bursting the ampules (40). In these cases, the sample materials must be ashed or digested prior to irradiation.

An alternative to ashing or digestion is extraction. Human adipose tissue and liver were analyzed for chlorinated hydrocarbons using NAA (41). The tissues were ground and extracted with organic solvents. After further treatment, the extracts were separated on an alumina column and the halocarbon fractions were placed in a polyethylene vial and irradiated directly.

Direct Analysis

Principle of the Technique--

Samples are irradiated and the resultant gamma-rays counted without any sample preparation except perhaps washing dust or other contaminants from the surface of the sample.

Biological Matrices--

Matrices which have been analyzed directly include hair and nails (31,35, 36,42-48), urine (36), serum (30) and tissue (kidney, liver, brain, and bone) (36).

Elements Analyzed--

Elements determined in hair and nails include Na, Mg, Al, S, Cl, Cr, Ti, V, Mn, Cu, Br, Sn, Ba, I, Hg, K, Sc, Co, Fe, As, Rb, Sm, and La (43). Additional elements determined by Perkons et al. (44) in hair include Si, Cr, Ni, Zn, Se, Ag, Sb, Cs, Ce, and Au. Hg and Se were determined directly in foods, blood, urine, hair and tissue samples by Rossi and Clemente (36). Versieck et al.

measured Mn, Cu, and Zn directly in serum (30). Tomza et al. (20) determined 24 elements (Na, Mg, Al, Cl, K, Ca, V, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Br, Ag, Cd, In, Sb, I, La, W and Au) in the hair of individuals working in a nonferrous smelter. As, Se, Ag, Cd and Sb were found to be elevated in this group relative to a group of controls. The only sample preparation was washing the hair in acetone and water as recommended by the IAEA. Turkstra et al. (21) determined 28 elements directly in hair using time delay to control interferences. In this analysis, problems of signal overlap were encountered with Ca, I, Mg, Mo, Se and V. In still another example, Houtman et al. (49) determined five metals (Cu, Zn, As, Sb, and Se) directly in hair while Cortes et al. (50) did the same for determination of As and Hg. Finally, Mangel and Gulati (51) determined Zn, Fe, Co, Cr, Se, Hg and Sb directly in 25 samples of hair taken from five persons.

Method Interferences--

The methods used to deal with interferences depend upon the type of sample and the element(s) being determined. Othman and Spyron (43), for example, used time resolution to determine 27 elements in hair. Na, Mg, Al, S, Cl, Cu, Ti, V, Mn, Cu, Br, Sn, Ba, I, and Hg were determined with 20 min of irradiation (2×10^{12} n/cm^{2}s), a 90 sec delay, and 20 min of counting. K, Sc, Cu, Fe, Br, As, Rb, Sm, Ba, La, Hg, and Au were determined with 30 h of irradiation, a 24 h delay and 3000 sec of counting. Perkons et al. (44) also used time resolution to analyze hair. Short-lived isotopes were determined with 5 min of irradiation and 1000 sec of counting; long-lived isotopes were determined with 38 h of irradiation, a delay of 168 to 200 h, and a counting time of 2-24 h. Heydorn, on the other hand, determined As in hair by direct irradiation, acid dissolution and then precipitation of the As with thioacetamide (10). McKinney et al. (52), in their determination of organically-bound chloride and bromide in human milk and blood serum, removed inorganic chloride and bromide by passing the samples through a molecular sieve column prior to irradiation. Mantel (53) in an effort to reduce interferences, developed a system for magnetic deflection of the beta particles being emitted from the irradiated samples. The result was a reduction of 85 to 99 percent of the background arising from P, K, Cl and Na.

Concentration Factor/LOD--

As the samples are analyzed directly there is no concentration factor. Othman and Spyron (43) reported detection limits for the direct analysis of 6 mg of nail as follows: Na-8 μg/g, Mg-12 μg/g, Cu-7 μg/g, and Mn-0.4 μg/g.

Recovery Data - Quantification Method--

Little work has been reported on recovery. McKinney et al. (52) used molecular sieve to remove inorganic chloride and bromide prior to determination of organically-bound chloride and bromide. Human milk samples spiked with PCBs and 2,4,5-T showed greater than 90 percent recovery using this desalting process while a recovery of 105±8 percent was achieved with p,p'-DDE. Levels measured provide some information about quantification. Rossi and Clemente (36) found levels of Hg and Se in environmental samples, foods, tissue and hair ranging from 1-100 ng/g, though Hg was found to be 3000 ng/g in the kidney of one subject. Perkons et al. (44) determined some 30 elements in hair with a broad range found for each; example means are Al-30.6 ppm, Cr-8.3 ppm, Se-3.7 ppm, and Sc-0.013 ppm.

The method of quantification used for analysis of untreated samples is the same as that used for all other types of samples.

Precision and Accuracy--

Very little precision and no accuracy data is presented in the literature sources utilized. Versieck et al. (30) measured Mn, Cu, and Zn in serum and reported values based on replicate analyses. There were ~13% (RSD) for Mn at ~0.5 μg/L, ~6% for Cu at ~1 μg/L and ~7% for Zn at ~ μg/L. Dutkiewicz et al. (23) analyzed 21 samples of hair made up from samples acquired from 210 children. The precision for the determination of each of 17 elements varied with the element and its level. Precision varied from ±30 percent for Cu and Ag to ±4 percent for Br.

Simplicity, Cost, and Sample Throughput--

Direct analysis of a sample provides some measure of simplicity and helps to control costs. It will also accelerate sample throughput.

Lyophilization

Principle of the Technique--

Samples are frozen and then placed in a specialized vacuum chamber. The water within the sample is sublimed from the sample leaving a dry, easy-to-handle sample.

Biological Matrices--

Lyophilization has been used for placental tissue (38), normal and psoriatic epidermis (37), maternal and fetal blood (38), urine (39), and tissue (54).

Elements Determined--

Baglan et al. (38) determined Se, Rb, Fe, Zn, and Cu in lyophilized placental tissue, and maternal and fetal blood. Molin and Wester (37) measured Br, Cd, Cr, Mo, Rb, Se, As, Au, Cs, Hg, La, Sb, Sc, Sm, and W in dried normal and psoriatic epidermis. Cornelis et al. (39) measured Na, Cl, K, Br, As, Cu, Mn, Zn, Ca, Co, Cr, Cs, Hg, Rb, and Se in lyophilized urine. Bencko and Symon (55) and Skerfing et al. (56) determined As and Hg in blood, respectively. Byrne and Kosta determined V in food, tissue, urine, feces, blood, milk, bile, hair, bone, and teeth (54). Behne and Jurgensen (24) determined 12 elements (Br, Na, Ag, Co, Cr, Cs, Fe, Rb, Sb, Sc, Se, and Zn) in lyophilized serum using time delay. Dermis and epidermis were oven dried and analyzed for Se, Cr, Fe, Co, Zn, Se, Rb, and Cs by Molokhia et al. (25,33) using time delay to control interference. Dang et al. (11) lyophilized, powdered and homogenized samples of human tissue and milk prior to determination of As. Following irradiation, though, the samples were acid digested. The arsenic was converted to $AsCl_3$, distilled away from the sample, collected in cold water, then converted to As_2S_3 with H_2S and finally counted. Human serum albumin was lyophilized for 72 hours prior to irradiation (Maenhaut et al., 19). The analysis for 18 elements involved both time and delay and chemical separation. Nakahara et al. (18) lyophilized blood serum prior to irradiation. Fourteen elements were determined by using the method of time delay while four were determined by destructive chemical separation because of interferences. Fourteen elements (K, Au, Cd, Cr, Cu, Mo, Ag, Co, Cs, Fe, Rb, Sb, Se, and Zn) were determined in platelets which had been dried into the forms of pellets (Kiem et al., 57).

Great care was taken in this study to retain the original integrity of the platelets during their separation from the original blood samples. Lin (32) reported on the determination of Cr, Co, Fe, Rb, Sc, Se, and Zn in 80 blood samples which had been lyophilized for 48 hours. The dried blood samples were powdered and irradiated for 30 hours at $2x10^{13}$ n/cm^2 s. Counting was done after a two week delay.

Method Interferences--

In their determination of Se, Rb, Fe, Zn and Co in placental tissue. Baglan et al. (38) irradiated 250 mg samples and then counted after several weeks in order to avoid interference by the short-lived ^{24}Na and ^{42}K species. Cornelis et al. (39) analyzed lyophilized urine samples using three different irradiation/decay/count programs. Na, Cl, K, and Br were determined after irradiation for 15 minutes; Cl was counted after 5 minutes of delay and Na, K, and Br after about 1 hour. As, Cu, Mn, and Zn were determined with an irradiation time of 3 hours and counted after delays of 2-24 hours. Finally, Br, Ca, Co, Cr, Cs, Hg, Rb, Se, and Zn were determined with an irradiation of 14 hours and a delay of one week to a fortnight. Because of their extremely low concentrations and high ^{32}P interference, Cr and Hg were isolated by distillation after irradiation. Lin (32), in his determination of seven elements in lyophilized blood samples, found that ^{46}Sc interfered with ^{65}Zn. To overcome this problem, Lin derived a correction factor from the 1120.3 Kev/889.4 Kev intensity ratio for ^{46}Sc. Nakahara et al. (18), turned to destructive chemical separation to determine 4 of 18 elements in blood serum because of interference from ^{24}Na and ^{82}Br. Following irradiation, the samples were acid-digested and then chemically treated so as to extract Mn, As, Cu and Zn as their APDC complexes into chloroform. In order to adequately minimize interferences from ^{24}Na, ^{42}K and bremstrahlung from ^{32}P, Molokhia et al. (25) delayed counting from 2 to 9 weeks for the elements Se, Cr, Fe, Co, Zn, Se, Rb, Ag, and Cs in oven-dried skin.

Concentration Factor - LOD--

Lyophilization usually does not reduce the bulk of tissue samples. It does, however, reduce the volume of liquids such as blood and urine, and thus allows larger samples to be irradiated. The concentration factor for these liquids could well be 10-100.

The detection limit was reported in three studies reviewed wherein lyophilization was used. Skerfing et al. (56) determined Hg in blood with a detection limit of about 0.5 ng/g. Parish et al. (58) determined As in blood and urine with a detection limit of 0.05 to 0.10 μg/g. When Byrne and Kosta (54) determined Vanadium in body fluids and tissues using wet ashing, a detection limit of 0.2 ng was achieved.

Recovery Data - Quantitation Method--

The only recovery data presented was that of Byrne and Kosta (54) who determined V in body fluids and tissues. They performed post-irradiation ashing, complexation and extraction prior to counting. The recovery for this procedure was 96%.

The method of quantification used with lyophilized samples is the same as that used for all other types of samples.

Precision and Accuracy--

Very limited precision and accuracy data was presented by the authors who employed lyophilization. Behne and Jurgensen (24) determined the precision for analysis of lyophilized blood serum to be less than 2 percent for Br, Fe, Na, Se, and Zn and less than 5 percent for Cs and Rb; the method of time delay was used to control interferences. Byrne and Kosta (54) checked the accuracy of their V analysis by analyzing NBS-SRM 1577. These authors measured 58 ng/g while 55-56 ng/g was expected.

Skerfing et al. (56) determined the precision of their analyses for mercury in blood cells and plasma. They reported the coefficient of variation to be 35% for analysis of a plasma sample containing 2 ng Hg/g (8 determinations) and 11% for analysis of a blood-cell sample without about 120 ng Hg/g (6 determinations).

Simplicity, Cost, and Sample Throughput--

Lyophilization is a relatively simple technique though it will add several days to the analysis time as the lyophilization process can be slow. The cost of lyophilization should be minimal.

Ashing/Digestion

Principle of the Technique--

Samples can be ashed or digested in numerous ways including acid digestion, Parr bomb digestion, dry ashing, and acid reflux/extraction.

Biological Matrices--

Versieck et al. (40) ashed serum samples in an oven for 24 hours at 100, 200, 350, and 450°C successively. Tracer experiments with ^{51}Cr and ^{60}Co showed there were no losses during this process. In their measurement of V in liver and serum, Cornelis et al. (12) found it necessary to complete a part of the chemical separation prior to irradiation because of the short half-life of the product isotope. The samples were lyophilized and dry-ashed in quartz at 450°C. Irradiation was then performed, the ash dissolved in 3N HCl and the V taken up as the cupferronate in chloroform. Cornelis et al. (13) used this same approach in the determination of V in human serum samples taken from 22 males and 18 females. Meesuk (59) reported on the analysis of 40 whole blood samples for Se, Cu, and As. The blood samples were irradiated and the product dissolved in hot HCl. Carriers were added and a separation performed which resulted in select precipitations of Se, CuSCN and As_2S_3. A complex procedure was used by Biso et al. (15) to determine mercury in food and hair. Irradiation was performed for eight hours at 2.1×10^{13} n/cm^2 s followed by a decay period of eight hours. The samples were then cooled in liquid nitrogen, transferred to a Bethge apparatus and digested in a mixture of nitric and sulfuric acids. Carrier was added and the excess nitric acid driven off. Hydroxylamine and ammonium hydroxide were added to precipitate elemental mercury. The mercury was then dissolved in nitric acid and precipitated with thiocyanate. The yield of this total process was reported to vary between 70 and 80 percent.

Elements Determined - Physical Chemical Properties--

Versieck et al. (40) determined Co and Cr in human serum. Janghorbani et al. (60) measured Zn in human blood using a complex procedure of acid-digestion, irradiation, extraction and precipitation. Variations in the process were used for red blood cells, plasma and feces. The process was complicated enough that only four samples per day could be analyzed. Woittiez et al. (14)

combined chemical separation and time delay to determine 12 elements in human serum. Total serum samples were acid-digested, the sodium removed using HAP, the sample irradiated and Mg, K and Cu measured. Following irradiation and acid digestion, ar, and Sb were converted to their hydrides, swept from the sample, collected on activated charcoal and counted. The elements Fe, Zn, Br, Rb and Se were determined using time delay to minimize interferences. Vanadium in the various protein fractions of blood was determined with partial chemical separation prior to irradiation (Sabbioni et al., 16). After separation from whole blood, the serum was fractionated using gel filtration. The protein fractions were acid-bomb digested and the metals separated on a Chelex 100 resin column. The vanadium-containing fraction was irradiated for 3 minutes at 5×10^{12} n/cm^2 s and then counted for 3 minutes.

Method Interferences--

Versieck et al. (40) irradiated the ashed serum samples for 12 days at a flux of ~10^{14} n/cm^2 sec. Interference from Fe was found to be negligible with the serum samples containing ~1.5 ng Fe/L. After a decay of 30 days, the Cr was separated by distillation as CrO_2Cl_2 because of ^{32}P interference. Sabbioni et al. (16) avoided interference from Na, Cl and Br when determining V in protein fractions of blood by separating them from the V on a Chelex 100 column following acid digestion and preceding irradiation. Interference from Na, Cl and Br in the determination of V in human serum was avoided by extraction of the V with cupferron in chloroform following dry ashing, irradiation, and dissolution in hot HCl (Cornelis et al., 13). Versieck et al. (34), in their determination of Mo in serum, eliminated bromine as an interference by volatization as HBr and avoided interference by sodium through extraction of the molybdenum with 8-hydroxyquinoline in chloroform. Cornelis et al. (13) and others described previously found it necessary to separate V from serum and blood samples in order to avoid interference from ^{24}Na, ^{38}Cl, ^{80}Br and ^{66}Cu.

Concentration Factor - LOD--

Versieck et al. (40) reported that ashing resulted in a four to five fold increase in sample size. No detection limits were presented. Mercury in urine was determined by Lo et al. (61) using lead diethyldithiocarbamate to both extract and preconcentrate the Hg. Wet oxidation was performed through the addition of HNO_3 and H_2O_2. Bromine was extracted with chloroform. After pH adjustment, the Hg was extracted with the lead diethyldithiocarbamate in chloroform. The chloroform was evaporated and the concentrated mercury-containing residue irradiated and counted. Cornelis et al. (13) who determined V in liver and serum using lyophilization, dry ashing, dissolution in HCl and extraction as the cuperferronate, reported lower limits of detection of 420 pg with 5 minutes of irradiation at 2.2×10^{12} n/cm^2 s and 40 pg with 8 minutes of irradiation at 8×10^{13} n/cm^2 s. When mercury was determined in hair and food using 8 hours of irradiation at 2.1×10^{13} n/cm^2 and ultimate collection as the thiocyanate, a limit of detection of 1 pg was achieved (Biso et al. (15)).

Recovery Data - Quantitation Method--

Versieck et al. (40) in their study of Cr and Co, tested recovery using ^{51}Cr and ^{60}Co. Recoveries were in the range of 95-110% though most ranged from 98-100%. The method of quantification used with ashed/digested samples is the same as that used for all other types of samples. Sabbioni et al. (16) used ^{48}V-labelled rat blood to determine recovery of V in protein fractions of blood. Following acid-bomb digestion, separation of metals with a Chelex 100 column, and irradiation, a recovery of 89 to 90 percent was achieved. When Biso et al. (15) determined mercury in hair and food using a process of irradiation, time delay, acid-digestion and ultimate collection as the thiocyanate a recovery of 70 to 90 percent was achieved. Janghorbani et al. (60) used a complex chemical separation procedure to determine Zn in human blood. The result was recoveries (tested by spiking with ^{70}Zn) determined to range from 47 to 100 percent. With a combination of time delay and separation through extraction with 8-hydroxyquinoline, mean recoveries of 97.7 and 98.8 percent were achieved in a determination of Mo in serum (Versieck et al., 34). A tracer study showed recovery to be 99 percent when mercury in urine was determined using a preirradiation separation scheme including extraction and preconcentration with lead diethyldithiocarbamate (Lo et al., 61).

Precision and Accuracy--

Versieck et al. (40) in their study of Co and Cr in serum, tested precision and relative accuracy. These workers analyzed six times the multielement serum standard of Cornelis et al. (13) (concentration of Cr-19.32 mg/kg; concentration of Co-6.46 mg/kg). They obtained values of 19.51 ± 1.38 mg/kg for Cr and 6.17 ± 0.23 mg/kg for Co. Furthermore, Versieck et al. (34) compared the results of two Cr determinations in human liver by their technique with the results obtained by a previously reported separation scheme. The Versieck procedure yielded values of 5.66 and 6.84 μg/kg wet weight while that of Lievens yielded 5.72 and 7.00 μg/kg wet weight. Duplicate analyses of serum using the Versieck procedure gave the following values: for Cr, s = 0.084 μg/L, and for Co, s = 0.051 μg/L. Lo et al. (61) reported a precision of ±7 percent for mercury at 31 ppb and ±10 for mercury at 7.1 ppb when using a preirradiation scheme including extraction and preconcentration with lead diethyldithiocarbamate.

Simplicity, Cost, and Sample Throughput--

Ashing or digestion complicates any procedure and increases the chances for loss and contamination. Costs for analyses will be increased from 10 to 100%, depending upon the approaches used to deal with interferences. Certainly ashing/digestion will add 1-3 days to the time required for analysis.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

NAA is a method of choice when a variety of elements are to be determined at low levels (<1 ppm). This is especially true when the list of elements to be determined includes Cl, Br, I, and metals such as Sm, Rb, Se, Ce, and Sc, which cannot be determined readily by alternate trace element techniques such as atomic absorption spectrophotometry. In order to achieve low detection limits with NAA, extensive delays in counting and/or separations may be necessary. These will raise analysis costs significantly. Sufficient data is not available to develop method/analyte correlation parameters. As noted in Table 5-8, various time delays and chemical separations have been used for the same elements in the same sample matrix. In summary, time delay seems used most often except where interference from Na, Cl, Br and Cu present a problem or the activation product is short-lived, e.g., V; then chemical separation is required.

TABLE 5-8. APPROACHES TO MEASUREMENT BY NAA

	Hair	Skin*	Blood*	Urine*	Tissue*
Mn	a		e	b	
Hg	b,c	e	e	c,e	e
Mo	b	e	e	e	e
Ni	b				
Nb	No data				
Pd	No data				
Pt					
K	a,b	a	a	a	a
Re	No data				
Rh	No data				
Rb	b,c	b,c	b,c	b,c	b,c
Ru	No data				
Sc	c	c	c		
Se	b,c	b,c	b,c	b,c	b,c
Si	No data				
Ag	c	c			
Na	a,b	a	a,b	a	a
Sr	No data				
Ta	No data				
Te	No data				
Tl	No data				
Sn	b				
Ti	a				
U	No data				
V	a	d	d	d	d
Zn	b,c	b,c	b,c,e	b,c	b,c
Al	a	a	a	a	a
Sb	b,c	b	b	b	b
As	b	e	e	b,e	e
Ba	c				
Be	No data				
Bi	No data				
B	No data				
Br	a,b	b	b	a,b	b
Cd	b				
Ca	a			c	
Ce	b,e				
Cs	c	c	c	c	
Cl	a	a	a	a	a
Cr	b,c	c	c,e	c	c
Co	b,c	c	c,e	c	c
Cu	a,b	e	e	b,e	e
Au	b				
Hf	c				
I	a				

(continued)

Table 5-8 (continued)

	Hair	Skin*	Blood*	Urine*	Tissue*
Ir					
Fe	b,c	b,c	b,c	b	b,c
La	b				
Pb	No data				
Li	No data				
Mg	a				
In	a				
Sm	b				
W	b				
Zn	c				
S	b				
Si	-				

[*] Normally lyophilized.

[a] Direct, short-time delay.

[b] Direct, medium time delay.

[c] Direct, long-time delay

[d] Pre-irradiation separation.

[e] Post-irradiation separation.

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SECTION 6

ELECTROCHEMICAL METHODS

Although several electrochemical methods have application to biochemical analysis, only two were cited in the data base "Chemicals Identified in Human Biological Matrices" and Chemical Abstract Data Base and have been included for review, namely potentiometry and voltammetry. Specifically, potentiometry involves ion-selective electrodes in conjunction with a pH meter and voltammetry (anodic stripping and cyclic) utilizes a three-electrode electrochemical cell controlled and monitored with a combination potentiostat function generator.

Other electrochemical methods such as differential pulse polarography, linear sweep voltammetry, and chromopotentiometry are analytical methods for the determination of electrochemically active species in aqueous complex matrices applicable to compounds of interest to this review. However, they are not specifically referenced in this section because no examples of analysis in human biological media were found.

ION SELECTIVE POTENTIOMETRY

Instrumentation

Principle of the Technique--

Ion selective electrodes allow measurement of the potential of unpolarized electrodes at zero current for specific analytes. The experimental procedure for their use is quite similar to that for a pH electrode. They are used with a reference electrode, such as saturated calomel or silver-silver chloride, and a high impedance potential measuring device such as a pH meter with a millivolt scale. By direct application of the Nernst equation, the activity of the analyte can be computed from the potential according to the equation:

$$E_A = E_A^o + \frac{RT}{nF} \ln (A)$$

where

E_A = cell potential of species, A

E_A° = standard reference half-cell potential for species, A

R = universal gas constant

T = absolute temperature

F = Faraday constant, 96,479 coulombs per equivalent

n = number of electrons transferred per formula unit of the reaction

A = activity of species, A

Calibration is achieved by plotting ln (A) vs. E_A.

The selectivity of the electrode is provided by the choice of electrode materials. For example, a fluoride selective electrode is made of a lanthanum fluoride crystal membrane doped with a rare earth element which presumably acts in such a way that only fluoride ions carry the current across the membrane. This electrode system is highly selective for fluoride; but at high pH, hydroxide ions constitute a major interference which limits its usefulness. The introduction of anion selective, precipitate-impregnated membrane electrodes has been an important development for selective ion electrodes. The membrane is made of a silicone rubber matrix which incorporates precipitated particles of silver halide or barium sulfate. The electrode is relatively insensitive to the nature of cations. The most selective and sensitive electrode system of this type is the silver iodide membrane electrode which responds to iodide concentrations as low as 10^{-7}, with relatively little interference from common ions.

Chemicals Analyzed--

Table 6-1 lists common selective ion electrodes as well as specific performance parameters for each sensor. It should be noted that each of these ion electrode systems is sensitive only to the ionic, inorganic forms of the element. Literature references have reported measuring fluoride and cyanide in biological matrices using selected ion electrodes.

Methods of Quantitation--

From the Nernst equation, it is apparent that potentiometric membrane electrode systems are sensitive to the activity of the electroactive species. In order to use such electrode systems for the determination of concentration rather than activities, it is important to consider the effects of ionic

TABLE 6-1. PROPERTIES OF ION-SELECTIVE ELECTRODES

Ion	Membrane	Lower Detection Limit	Principal Interferences
H^+	glass	10^{-14} M	none below pH 13
Na^+	glass	10^{-6} M	H^+, Ag^+
K^+	glass	10^{-6} M	H^+, Ag^+, Na^+
NO_3^-	liquid	10^{-5} M	I^-, Br^-
Cl^-	liquid	10^{-5} M	I^-, NO_3^-, Br^-
Ca^{++}	liquid	10^{-5} M	Zn^{++}, Fe^{++}, Pb^{++} Cu^{++}
Water hardness	liquid	10-8 M	Zn++, Fe++, Pb++
F^-	solid	10^{-6} M	OH^-
Cl^-	solid	5×10^{-5} M	$S^=$, I^-, Br^-, CN^-
Br^-	solid	5×10^{-6} M	I^-, $S^=$
I^-	solid	5×10^{-8} M	$S^=$
$S^=$	solid	10^{-17} M	Hg^{++}
CN^-	solid	10^{-6} M	$S^=$, I^-
Ag^+	solid	10^{-17} M	Hg^{++}
Cd^{++}	solid	10^{-7} M	Ag^+, Cu^{++}, Hg^{++}
Pb^{++}	solid	10^{-7} M	Ag^+, Cu^{++}, Hg^{++}
Cu^{++}	solid	10^{-8} M	Ag^+, Hg^{++}, $S^=$

strength on the activity coefficient of the electroactive species. To avoid the uncertainty of estimating an activity coefficient, it is useful to determine the effect of an added known amount of the test species on the potential or to adjust the ionic strength of the sample to that of the standard solution. A constant ionic strength can be obtained by using a "swamping electrolyte." This technique frequently referred to as the "ionic medium" method has been used to calibrate potentiometric membrane electrode systems.

Quantitation of fluoride ion was achieved by preparation of a standard curve (1,2) or by the method of additions (3). Calibration curves for F^- were generated by the addition of fluoride standard to fluoride-free water (2) or, if serum was the matrix being analyzed, to human serum drawn from an individual who had not ingested fluoridated water within 24 hours (2). Canine serum and isotonic saline have also been used as surrogate matrices for calibration (2). Cyanide was quantitated by McAnnalley et al. (4) by application of a standard curve.

Limit of Detection--

Detection limits for the various selected ion electrodes are included in Table 6-1.

Precision--

Coefficients of variation (CV) for the determination of fluoride with an ion selective electrode were in the range 1.3 to 5.6%. Hanhijärui et al. (5) reported coefficients as high as 16% near the limit of detection.

Accuracy--

Determination of fluoride spiked directly into plasma and urine and, subsequently analyzed with a fluoride selective electrode gave recovery values greater than or equal to 90% (1,3,5,6). Significantly lower recoveries (70-80%) were observed only near the limit of detection (LOD).

Simplicity, Cost, and Sample Throughput--

Ion selective electrodes are very easy to use and inexpensive compared to other techniques such as AA. This method, however, is sensitive only to labile (noncomplexed), ionic species and, consequently, determination of the inorganic ions is quite facile. Organic or organically-complexed forms require preliminary extraction or digestion which makes the analysis considerably more labor intensive.

Sample Preparation Methods

Microdiffusion Procedure--

Principle of the technique--Organic fluoride (e.g., methoxyflurane) or cyanide is converted to the corresponding inorganic species by diffusion in a multi-chamber petri dish from an acidified sample to an alkaline trap (4,7,8). Fluoride or cyanide is then determined directly in the alkaline medium with the appropriate ion selective electrode.

Biological matrices/chemicals analyzed--Organically- bound fluoride was determined in blood (7,8) and urine (8). Organic cyanide compounds were measured in blood (4).

Method interferences--No interferents to the determination of fluoride were identified in the citations of this data base. Sulfide interference in the analysis of cyanide is removed by the addition of lead acetate to form insoluble lead sulfide (4).

Concentration factor - LOD--This method of sample preparation does not enhance the analyte concentration. The LOD is identical to the instrumental LOD.

Recovery data--No recovery data was presented for fluoride or cyanide ions.

Precision and accuracy--Precision and accuracy were not addressed.

Simplicity, cost, and sample throughput--McAnnalley et al. (4) estimated that cyanide ion could be determined in blood using an ion selective electrode following microdiffusion at a rate of approximately 1 sample per hour. Since the approach to the determination of fluoride is essentially identical, this estimate should apply to fluoride as well.

Direct Measurement--

Principle of the technique--Inorganic fluoride is determined directly in the matrix after addition of pH 5 acetate buffer or after the sample is diluted 1:1 with total ionic strength acetate buffer (TISAB). During the analysis of fluoride ion pH control is very important. Since the fluoride ion is a weak acid (pKa ~3), the undissociated acid will be present at low pH and the fluoride electrode will not respond to this species. Under basic conditions, hydroxide ion will interfere with the analysis.

Biological matrices/chemicals analyzed--Blood (2,3,6,8-10) and urine (4,8,9,11,12) were analyzed for fluoride ion by this method.

Method interferences--Tissue debris was identified as an interferent by Speaker, particularly at low fluoride concentrations (8).

Concentration factor/LOD--The direct measurement method does not enhance the analyte concentration. The method LOD is identical to the instrumental LOD.

Recovery data/quantitation method--Recoveries were determined by spiking fluoride directly into the matrix or into a surrogate matrix followed by measurement with a fluoride selective electrode. Recoveries ranged from 90 to 100% (1,3,5,6), although lower values (70-80%) were reported near the limit of detection (5).

The results were quantitated using either a calibration curve (1,2), method of additions (3), or Gran's plot (2).

Precision and accuracy--Precision was found to be in the range 1.3-5.6% relative standard deviation in the 10-30 ng F^-/mL range (3,6,9), but was as high as 16% near the LOD (5).

Simplicity, cost, and sample throughput--Since the direct measurement of fluoride in body fluids with a fluoride selective electrode requires only the addition of a buffer prior to the measurement of the electrode potential, sample throughput should be very high (~6 samples per hour). Consistently, sample analysis cost should be low.

ANODIC STRIPPING VOLTAMMETRY (ASV)

Instrumentation

Principle of the Technique--

Anodic stripping voltammetry involves two consecutive steps: (a) the electrolytic separation and concentration of the electroactive species to form a deposit or an amalgam on the indicator electrode, and (b) the dissolution (stripping) of the deposit in which the component is determined. The separation step, or pre-electrolysis step, may be performed quantitatively or on a reproducible fraction of the electroactive species. This can be done by performing the pre-electrolysis step under carefully controlled conditions of potential, time of electrolysis, and hydrodynamics of the solution. The deposition under reproducible stirring conditions follows the first order kinetic expression

$$Q_t = Q_o (1 - e^{-kt})$$

where Q_t is the number of coulombs in the stripping peak at any time t, Q_o is the number of coulombs equivalent to the total metal ion concentration in the sample solution, t is the deposition time (sec), and k is the rate constant. The rate constant, k, is equal to

$$k = \frac{DA}{V\sigma}$$

where D is the diffusion constant of electroactive species in the test solution in cm^2/sec, A is the electrode surface area in cm^2, V is the volume of the solution in cm^3, and σ is the effective thickness of diffusion layer in cm.

Ordinarily partial deposition of a sample is sufficient for analysis. Under conditions where the rate constant is not reproducible, longer plating times increase the accuracy. With a long deposition time, the expression e^{-kt} approaches zero, therefore, slight changes in k which may occur between analyses have little effect on the precision of the results.

Quantitative determination of the deposited materials is made in the stripping step, most commonly by a chronoamperometric technique with a linear potential sweep. Commerical polarographic equipment employing operational amplifiers may be used for slow potential sweep rates, in the order of 2 to 100 mv/sec. When the proper electrode is used under controlled conditions, the concentration of metals in solution is linearly proportional to the peak current, and qualitative identification of the metal ions in solution is obtained from peak potential values.

The stripping step is usually done in an unstirred solution by applying a potential, either constant or varying linearly with time, of a magnitude sufficient to drive the reverse electrolysis reactions. Quantitative determinations are done by integrating the current-time curves (coulometry at controlled potential) or by evaluating the peak current (chronoamperometry with potential sweep). Several modifications of the separation and stripping steps have been reported.

Hanging-drop mercury electrodes of the Gerischer's or Kemula's type have been widely used for anodic (or cathodic) stripping analysis (20). Greater sensitivity has been achieved by use of electrodes which consist of a thin film of mercury on a substrate of either platinum, silver, nickel, or carbon.

Errors due to nonfaradaic capacitance current components can be minimized by proper choice of stripping technique.

Chemicals Analyzed--

ASV has been successfully applied to the analysis of Ag, Au, Ba, Bi, Cd, Cu, Ga, Ge, Hg, In, K, Mn, Ni, Pb, Pt, Sb, Sn, Tl, and Zn ions. Literature references report the analysis of zinc, cadmium, lead, and copper in human biological media. This technique is sensitive to a wide variety of redox species including metals, organic acids, phenols, amines, quinones, etc.

Methods of Quantitation--

Methods of quantitation of analytes measured by anodic stripping voltammetry are usually performed by methods of addition.

Limit of Detection--

Limits of detection for ASV usually range from 10^{-7} to 10^{-10} M. A limit of detection of 1 ppb was reported for Cd by ASV (13). Zinc, lead, and copper have LODs approximately equivalent to that for cadmium.

Precision--

Reproducibility reported for ASV analysis of Cd, Pb, Zn, and Cu was approximately 10% (13,14), although Hammond et al. (15), and Chittleborough and Steel (16) reported CVs as low as 3%. A relative standard deviation of 2.6 to 13% was reported (20) depending on the metal and sample source.

Accuracy--

Lead was determined in blood within 10% accuracy by Hammond et al. (15), using ASV. Recovery from spiked samples was reported (20) in the range of 91 to 101%.

Simplicity, Cost, and Sample Throughput--

Anodic stripping voltammetry is an easy technique to implement and the analysis is rather fast. It is applicable to a wide variety of ionic species (metals and organics) and redox complexes. Costs of equipment for anodic stripping voltammetry are usually higher than for selective ion electrodes, but are still rather inexpensive and suitable for simultaneous determination of several metals (20).

Sample Preparation Methods

Only one citation in the data base for ASV made reference to sample preparation. Hecker et al. (13), determined Cd, Cu, and Pb in blood, urine, and hair by perchloric acid digestion followed by ASV using a thin-film Hg electrode. Relative standard deviation was less than 10% using this method. Presumably, although not documented, some type of sample digestion was employed in all cases where ASV was used, particularly where solid or very complex tissues (e.g., urine, teeth, blood, hair) were analyzed (13,14,16,18,19,20).

CYCLIC VOLTAMMETRY

Instrumentation

Principle of the Technique--

Rapid-voltage-scan techniques in which the direction of voltage scan is reversed are called cyclic techniques. In these techniques a ramp is applied over the full voltage-scan range and then reversed so that a descending ramp returns, almost invariably to the original potential. The scan rate in the forward and reverse directions is normally the same, so that the excitation waveform is actually an isosceles triangle. Cyclic voltammetry is that voltammetric technique in which the current that flows in a system is measured as a function of time and in which the excitation signal is a triangular potential. The potential is normally that of an isosceles triangle, although this is not strictly necessary. Cyclic voltammetry can be used in single-cycle or multicycle modes, depending upon the electrode, the reaction in question, and the information sought. In most cases the first and later scans are not identical. Multicyclic techniques have not been used for quantitative measurements because quantitative theory is not available for them. They are, however, useful diagnostic tools when complex mechanisms are involved. The measured parameters in cyclic voltammetry are the anodic and cathodic peak potentials $E_{p,c}$ and $E_{p,a}$; the anodic and cathodic peak currents $I_{p,c}$ and $I_{p,a}$; and the half-peak potentials, which are the potentials $E_{p/2,c}$ and $E_{p/2,a}$ at which the cathodic and anodic currents reach half of their peak values. The independent variables are the voltage scan rate and the range of potential over which the scan is made. The former is the most important parameter in a diagnostic sense, although proper selection of scan range often can eliminate interference from other processes.

Chemicals Determined--

Cyclic voltammetry should be applicable to the same chemicals as anodic stripping voltammetry. Specifically, cyclic voltammetry has been used for the determination of p-amino-diphenylamine in blood (21).

Methods of Quantitation--

The method of standard addition and calibration curves have been used for quantitation as reported by dePeuriot et al. (21).

Limit of Detection--

Not available from the reviewed literature, although it is estimated as higher than ASV.

Sample Preparation Methods

For the determination of *p*-amino-diphenylamine, blood samples were used with no further treatment. In the same work, human plasma was prepared by centrifugation at 2000 g for 20 min at 4°C and kept frozen at -20°C before use. No further details were given (21).

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

Single methods are presented in these citations for each analyte. Fluoride and cyanide were determined using an ion selective electrode. Zinc, cadmium, lead, and copper were measured by anodic stripping voltammetry. Cyclic voltammetry can be used with metal ions as well as organic compounds which can undergo redox reactions such as aliphatic and aromatic amines, nitro derivatives and quinones. Application of the fluoride and cyanide selective ion electrodes requires that these species be in the most reduced form. Conversely, determination of zinc, cadmium, lead, and copper by ASV is facilitated by oxidized elemental states. The applicability of the voltammetric methods in aqueous matrices is limited to those chemical species which have a redox potential between the limits of oxidation of water and reduction of hydrogen ion. In addition, the working electrode material, the electrolyte and the pH of the solution will influence the experimental limits. For a more detailed discussion see reference 22.

Simplicity, Cost and Sample Throughput--

Same as anodic stripping voltammetry.

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SECTION 7

EMISSION SPECTROSCOPY

INDUCTIVELY COUPLED ARGON PLASMA

Principle of the Technique

Emission spectroscopy is based upon the emission of electromagnetic radiation, generally in the ultraviolet and visible region, by atomic particles. In order to obtain an emission spectra, it is necessary to atomize the sample. In this process, the constituent molecules are decomposed and converted to gaseous elementary particles. An emission spectrum of an atomized element consists of a relatively limited number of discrete lines at wavelengths that are characteristic of that element.

There are several methods of atomizing the sample. Table 7-1 summarizes the various emission spectroscopic methods classified according to atomization method.

For electrical excitation sources, the distribution of energy levels for an atom or ion may be described as the Maxwell-Boltzman expression:

$$\frac{N_\mu}{N_o} = \frac{g_\mu}{g_o} e^{-(E_\mu - E_o)/kT}$$

where N_μ is the number of particles in the exited state; N_o is the number of particles in the bound state; g is the statistical probability of a particle being in the given state; E is energy; T (temperatures) is the equilibrium temperature of the discharge; and k is the Boltzman constant. This distribution (N_μ/N_o) is uniquely related to temperature and to the composition of gaseous particles.

Emission spectroscopy using a silver-argon DC arc has been applied to the measurement of trace elements in biological samples, using a static argon chamber where air is excluded from the system to eliminate cyanogen bands and

TABLE 7-1. CLASSIFICATION OF EMISSION SPECTRAL METHODS

Type of Spectroscopy	Method of Atomization
Arc	Sample heated in an electric arc
Spark	Sample excited in a high voltage spark
Argon plasma	Sample heated in an argon plasma
Atomic or flame emission	Sample solution aspirated into a flame
X-Ray emission or electron probe X-ray	None required; sample bombarded with electrons

background continuum. Further reduction of background has been achieved by replacing air with argon at subatomospheric pressure. This technique is more commonly known as inductively coupled argon plasma (ICAP) spectroscopy. Low argon pressure also results in a wider, more stable, positive arc column. Major improvement in arc stability and reproducibility of analyses can be achieved by the introduction of a tantalum sphere at the tip of a graphite counter electrode. Recently, hydride generation methods have been developed for ICAP spectroscopy with improved detection limits (1).

Chemicals Analyzed

ICAP is applicable to a wide range of elements including the ones listed in Table 7-2. Citations from the Chemical Abstract Data Base reported analyzing various metals such as: As (1), Bi (1), Sb (1), Se (1,7), Sn (1), Ca (7), Te (1), Zn (5-8), Cd (7), Mn (7,8,16), Si (7), Be (8), Hg (8), Cu (7,8), Co (8), Mg (7), Tl (8), Mo (8), Mn (7), Fe (7,8), P (7), Ti (8), V (8), Cr (4,7,9), Li (10), Sr (10), Ni (16), K (7).

Method of Quantitation

Quantitation is achieved by the method of standard addition. Procedures based upon that proposed by the U.S. Environmental Protection Agency (Fed. Register 44:69559-69564, 1979), have been used together with data analysis and statistics Biomedical Computer Programs (BMCP) packages have been used (7).

Limit of Detection

In general, the technique can detect nanogram quantities of various elements as illustrated in Table 7-3.

Some limits of detection (LOD) have been reported to vary with sample matrix (1), Table 7-2 illustrates this effect. Other LODs for ICAP spectroscopy are reported in reference (7), based on previously reported research.

Accuracy and Precision

Reported (1) precision data is shown in Table 7-4. Recovery of metals from urine samples has been reported (1) as an indication of accuracy. Table 7-5 shows this information.

TABLE 7-2. DETECTION LIMITS

Element	Wavelength Å	Lower Limit of Detection	
		Percent	Micrograms
Ag	3280.68	0.0001	0.01
As	3288.12	0.002	0.2
B	2497.73	0.0004	0.04
Bi	-	-	~0.1
Ca	3933.66	0.0001	0.01
Cd	2288.01	0.001	0.1
Cu	3247.54	0.00008	0.008
K	3446.72	0.3	30
Mg	2852.12	0.00004	0.004
Na	5895.92	0.0001	0.01
P	2535.65	0.02	0.2
Pb	4057.82	0.0003	0.03
Sb	-	-	~0.2
Se	-	-	~0.2
Si	2516.12	0.0002	0.02
Sn	-	-	~1
Sr	3464.45	0.02	2
Ti	3372.80	0.001	0.1
Tl	-	-	~0.1
Zn	3345.02	0.003	0.3

TABLE 7-3. INSTRUMENTAL DETECTION LIMITS[a] (ng/mL) FOR STANDARDS IN 20% HNO_3 AND NORMAL URINE

Element	20% HNO_3	Urine
As	100	2-20
Bi	500	1-50
Sb	80	1-15
Se	300	2-20
Sn	100	2-25
Te	600	1-30

[a] Detection limits correspond to the analyte concentration required to give a net signal equal to three times the standard deviation of the background.

TABLE 7-4. RELATIVE STANDARD DEVIATION OF ELEMENTS MEASURED IN URINE

Element	Measured (μg/L)	% RSD
Sb	2.6	26
Bi	4.5	27
Sn	<1.2	-
As(III)	9.9	27
Se(IV)	3.0	25
Se(VI)	7.0	18
Te(IV)	10	21
Te(VI)	<0.04	-

TABLE 7-5. RECOVERY OF METALS FROM URINE SAMPLES

Element	Added (µg/L)	% Recovery
Te(IV)	10	98
	20	105
	30	103
Te(VI)	10	92
	20	124
	30	118
Se(IV)	10	108
	20	90
	30	86
Se(VI)	10	88
	20	95
	30	105
Bi(III)	5	80
	10	88
	15	99
Sb	10	89
	20	91
	30	100
As(III)	10	92
	20	95
	30	94
Sn	10	111
	30	99
	50	83

Simplicity, Cost and Sample Throughput

ICAP is a technique ideally suited for multiple element analysis. It is very fast, simple and efficient. The cost of instrumentation is comparable to the cost of standard AA instrumentation.

OTHER EMISSION SPECTROSCOPIC TECHNIQUES

Principle of the Technique

Energy dispersive X-ray fluorescence (EDXRF) has been included under the broad category of emission spectroscopy (2). This is a technique for rapid, simultaneous multi-element analysis. When excited by an appropriate source, a sample will emit X-rays of energies that are characteristic for the elements composing the sample. By measuring the energies of X-rays that are emitted from an excited sample and counting the number of X-rays of each energy, EDXRF allows the identification of the elements present in the sample, and also allows determination of the relative concentration of each of the elements present.

The elements composing the sample are excited by an X-ray source which removes tightly-bound electrons from the innershell orbitals of the atoms. A "hole" is thus created in the shell of the excited atom, and the atom will immediately relax back to a more stable ground state by moving outer-shell electrons to fill the hole. When the electron moves to the inner-shell a quantum of energy is released equal to the difference between the original binding energy in the outer shell and the new binding energy in the inner shell. The electromagnetic radiations produced are emitted from the excited atom and can be used to identify the element in the sample, since each element produces X-rays of characteristic energies. These characteristic X-rays of the elements vary in a systematic and predictable manner according to the atomic number of the excited element.

The X-rays emitted from the excited sample strike a semiconductor silicon drifted lithium detector. This detector crystal is kept under vacuum and at liquid-nitrogen temperature to reduce thermal noise. X-rays enter the detector through a thin beryllium window, ionizing the semiconductor to create electron-hole charge pairs which are collected by the applied bias voltage to form a pulse. This pulse is fed to a preamplifier which converts the charge pulse into a voltage pulse to be further amplified and shaped in the Pulse Processor. The Energy-to-Digital Converter accepts this pulse, measures the peak amplitude (voltage) and converts it into a discrete numerical value that is proportional to the amplitude of the pulse from the Pulse Processor and hence to the energy of the incident X-ray. The voltage pulses are encoded into numerical values ranging from 0 to 1023, and sorted according to amplitude, which is proportional to the energy deposited in the detector by the incident X-rays. For each pulse converted, one count is stored in an appropriate channel of data memory, each channel representing a narrow slice of the total energy spectrum. These counts stored in memory represent the measured X-ray energy spectrum and are displayed as a histogram (bar graphs) of the number of X-ray counts measured (Y-axis) as a function of X-ray energy (X-axis) in KeV. The elements that are present in a sample can be identified by the location of their energy peaks along the horizontal axis. Since the number of X-rays produced is proportional to the number of atoms present in a sample, quantitative elemental concentrations can be determined from the intensities of the energy peaks.

Chemicals Analyzed

Emission spectroscopy including EDXRF is applicable to a wide range of elements, including those listed in Table 7-2. Literature reports included analyzing arsenic, as As_2O_3 and dimethyl arsenic acid (3).

Citations from the Chemical Abstract Data Base reported analyzing numerous metals (2). In addition, a number of carbon-based compounds have also been reported; they include cyanide (11), organophosphates (12), benzo[a]pyrene derivatives (13), acetaldehyde (14), phenolic substances (15), alcohols (2) and chloro-organic compounds (16).

Methods of Quantitation

Quantitation is achieved by the method of standard additions. Dilute standard solutions were added to sample aliquots prior to drying and ashing.

Limit of Detection

In general, the technique can detect nanogram quantities of various elements as illustrated in Table 7-3.

Accuracy and Precision

The mean relative standard deviation for quantities of chromium (4) ranging from 1-7 ng was 6% for triplicate analysis.

The precision of Li and Sr determination in blood serum has been reported (10) as 6% RSD at the 10 ng/mL level.

Simplicity, Cost, and Sample Throughput

These techniques do not appear to be simple in comparison to other analytical methods.

SAMPLE PREPARATION METHODS

Low Temperature Ashing

Principle of the Technique--

The low temperature ashing method is based on the low temperature burning of organic matter followed by acid dissolution of the remaining inorganic material. This method is commonly used for oxidizing and removing organic material in samples prior to analysis. Elimination of organic material results in improved accuracy and reproducibility of analyses. The preconcentration factor is an additional feature which is advantageous for certain materials. Organic material is oxidized in the oxygen plasma of a low temperature asher; this procedure not only minimizes the risks of contamination or loss of trace elements but yields a sample which is ideal for subsequent processing.

Ashing of serum, urine, and hair samples is rapid and complete, but complete oxidation of red blood cells and solid tissues including liver and muscle is more difficult.

Biological Matrices--

The biological matrices analyzed by emission spectroscopy after low temperature ashing include blood (6), plasma (5), urine (2,16), hair (5).

Chemicals Analyzed--

Citations in the original data base reported the analysis of arsenic, chromium, and zinc (5,6) after low temperature ashing. However, the method is applicable to many other elements.

Method Interferences--

No available data.

Limit of Detection--

The reported LOD for arsenic in blood using low temperature ashing was 1.2 pg/mL (17). Other LODs were not reported for this preparation method.

Recovery Data/Quantitation Method--

Mean recovery of chromium added to 20 serum samples prior to ashing was 99.7%. A radiolabeled chromium solution (50 µL) was also added to the top of hair samples in the ashing boats, and mean recovery for ten samples, in this instance, was 98.1%. Recoveries of sodium (101 ± 2% RSD) and potassium (103 ± 2%) from serum samples were equally satisfactory.

Precision and Accuracy--

Relative standard deviation of recovery of chromium added to 20 serum samples prior to ashing was 3.2%. RSDs for samples spiked with chromium (2%), sodium (2%) and potassium were equally good.

Simplicity, Cost, and Sample Throughput--

See discussion in Section 4.

Concentration and Separation Using Chelating Resins

Principle of the Technique--

Concentration and separation of elements and compounds prior to analysis is based on the chelating properties of some synthetic resins such as poly(dithiocarbamate), poly(acrylamidoxime) (9) and Amberlite and Dowex (15) at different pH values. For batch tests, pH-adjusted analysis are placed together with appropriate quantities of resin in precleaned polyethylene bottles and shaken for up to 24 hours. The resin is then separated from solution by filtration and analyte concentrations in both filtrate and acid-digested resin are determined.

Biological Matrices--

The biological matrices analyzed by emission spectroscopy after concentration and separation using chelating resins include urine (8,9) and dextrose (9), and human serum (15).

Chemicals Analyzed--

Metals such as Be, Zn, Mm, Hg, Cu, Co, Tl, Mo, Fe, Ti and V (8) and Cr(VI) and Cr(III) (9) have been reported. In addition, phenolic compounds (15) have also been studied.

Method Interference--

No available data.

Limit of Detection--

Detection limits for Cr are reported (9) as 140 ng/L and 1.4 µg/L depending on the resin.

Precision and Accuracy--

The accuracy and recovery of this method has been reported (8) to be pH dependent and resin dependent as well as element dependent. Except for V, the metals studied are quantitatively recovered either directly from urine or from acid-digested urine. The precision for Cr(III) was reported as 21% and 12.3% RSD (9).

Simplicity, Cost and Sample Throughput--

See discussion in Section 4.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETER

Emission spectroscopy is most applicable to the analysis of biological matrices containing inorganic compounds and elements.

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SECTION 8

PROTON INDUCED X-RAY EMISSION (PIXE)

INSTRUMENTATION

Principle of the Technique

Proton induced X-ray analysis (PIXE) is based on the measurement of X-rays emitted by a sample under bombardment of high energy protons. This technique is used for multielemental analysis of large number of samples in routine experiments (1,2).

X-ray fluorescence (X-ray-induced X-ray emission) has been used as an analytical tool for some time. However, it is only in recent times that X-ray emission caused by particles, such as alpha particles and protons, has been used for analytical purposes. The potential of the particle method arises, in part, from the fact that the cross section for characteristic X-ray production is quite large using particle excitation and that a single particle can give rise to several characteristic X-rays as it penetrates a sample.

There are several components to the method of particle induced X-ray emission (PIXE), including particle generation, X-ray production, and X-ray detection. The alpha particles and protons are generated by stripping the electrons from helium and hydrogen, respectively, in a plasma. The charged particles are then passed through a cyclotron or a Van de Graff accelerator such that they achieve extremely high kinetic energy (30-80 million electron volts for alpha particles and 2-4 million electron volts for protons). Using electromagnetic fields, the charged particles are directed into a narrow beam of 1-10 mm diameter and several microamperes intensity. The beam intensity is reduced to 1-100 nanoamperes by passage through a metal filter and then directed into the sample.

The samples can have a variety of forms. Liquids such as blood and urine are placed on thin sheets of plastic such as polystyrene or Mylar® (General

Electric) and brought to dryness in air or freeze-dried. Solids such as hair are attached to the film using adhesive. Tissue sections are normally freeze-dried and then attached to the plastic film. Maximum sensitivity is achieved if the sample is ashed or digested and the resultant solution is placed on the film and taken to dryness. This not only concentrates the sample but also minimizes the X-ray background arising from the carbonaceous sample matrix. The samples mounted on the plastic film are placed on a sample rack and then placed in a chamber in line with the particle beam. The chamber is evacuated to a pressure of about 5×10^{-5} Torr and the beam is directed into a sample.

When the particles strike the metal atoms within the sample, they knock out inner shell electrons. When upper shell electrons fall into these vacant orbitals, X-rays are released. The energy of each X-ray is characteristic of the element from which it is emitted. The numbers of X-rays emitted at a particular energy from a sample is a measure of the concentration of the emitting element. The X-rays are usually detected with a solid state detector such as a lithium-drifted silicon detector [Si(Li)]. When an X-ray passes through this detector, it creates free charge carriers (electrons and "holes"). The number of free charges is proportional to the energy of the X-ray. A voltage applied across the detector "sweeps" the charge from the detector as a pulse of electrical current. Using appropriate electronics, the pulse of current is converted to a digital signal. A computer keeps track of the numbers of digital signals of each magnitude. Digital signal magnitude is proportional to X-ray energy. The result is an X-ray spectrum of numbers of X-rays versus X-ray energy. The X-ray emissions from each element show up as peaks.

The use of the particle induced X-ray emission (PIXE) technique with nuclear microprobes has become an important tool for determining distributions of elements, across a specimen (3). The results are often represented by plotting the number of X-ray counts versus the distance along the sample. The energy loss of the beam when it passes the target can be used for estimating the amount of material being hit. It has been shown that the energy loss can be measured very accurately with the aid of the strong intensity of γ-rays from the $^{27}Al(p,p'\gamma)^{27}Al$ reaction induced by the tranversed beam in an Al beamstop. A semiempirical method for concentration assignment has been formulated and tested. The method is limited to thin targets and makes use of an initial beam energy of about $E_p = 3$ MeV. Since this energy is a reasonable compromise between minimal detection limits for the lighter and heavier elements, it is not a serious drawback. Samples of biological material have been tested, but in principle the presented method can be applied to other target materials.

Chemicals Analyzed

A wide range of elements can be analyzed with this technique. The elements normally measured using PIXE range from sodium (Z=11) to uranium (Z=92). The energies of the X-rays detected vary from 2 keV to 20 keV. The net response of a PIXE system to X-rays of varying energy is not linear, but peaked. With a proton system, sensitivity is at a high with calcium, manganese, and cobalt and low with sulfur, chlorine, molybdenum and cadmium. Among the elements of interest reported in the literature references are cadmium, lead, zinc, calcium, titanium, manganese, nickel, and strontium.

Elements such as Cl, K, Ca, Ti, Mn, Fe, Cu, Pb, As, Se, Zn, S, Ti, Ba, Br, Rb, Sr, Y have been analyzed in hair (3-9); similar elements have been analyzed in blood, plasma and serum (11,15,17,18,19,20,21). In addition, Ni, Cu, and Zn have been analyzed in albumin and P, Cu, Ca, K, S, Cl and Zn in skin.

Methods of Quantitation

Calibration plots are prepared from standard solutions. The linearity has proven to be very good over 4 orders of magnitude and the effects of X-ray absorption or proton energy loss in the sample are negligible. Internal standards are used to check that no rapid initial loss occurs when volatile species are subject to vacuum (14,16).

Yttrium in the form of Yttrium nitrate has been used as internal standard (12,18), samples were spiked with 820 μg/g of Y since it does not appear naturally in hair. Strontium nitrate has also been used as an internal standard (19).

Interferences are usually encountered when X-ray emission lines of one element overlap with the main line of the element of interest. Sophisticated computer software is used to remove the interfering line provided the adequate line ratio is determined.

Limit of Detection

The detection limits for multielement samples in a 10 minute bombardment are in the nanogram range. A lower limit of detection of approximately 200 picograms in the irradiated area has been reported with the more responsive elements when they are deposited on very thin plastic films. The detection limits for most metals of interest range from 0.1 to 1 ppm in biological fluids and tissue. The ultimate sensitivity available depends not only on the excitation technique, but also on the nature of the sample being analyzed and the spectral background. The main source of background in the case of particle excitation is bremsstrahlung originating from within the target due to stopping of electrons which have been knocked out of the target atoms by the incident protons. This background, which consists of a broad continuum of X-rays with a maximum at the low energy end of the spectrum, sets the lower limit of detection. Bremstrahlung is at a minimum with digested/ashed sample solution on a thin plastic film and a maximum with slices of freeze dried tissue. Another limitation with thick targets is that X-ray production efficiency varies from the front to the back of the sample. Any target having a real density of greater than 10 mg/cm^2 appears as an infinitely thick target to 3 MeV protons. Correction factors can be calculated and used when determining metal concentrations in thick targets; however, in order to calculate these factors, the major elemental composition of the target must be known, i.e., percent carbon, oxygen, iron, etc.

Some trace elements can be measured at the ppm level in a reasonable time (6). The detection limit of chromium has been reported (20) as approximately 0.3 ng/mL. LODs for other trace elements are reported (9) in the μg/g concentrations. The sensitivity for cadmium was found to be poor (12); however, 48-57 μg/g of Cd were reported. Limit of detection for lead and zinc in wet whole blood have been found (17) to be approximately 0.3 and 0.1 ppm, respectively, and for solidified (freeze-dried) blood are 2.0 and 0.7 ppm, respectively. For Fe, Zn and Sr the limit of detection in plasma were estimated (21) to be 0.08 ppm fo Fe, 0.065 ppm for Zn and 0.12 ppm for Sr, low temperature ashing improved these limits of detection by a factor of three, but reduced the precision and resulted in loss of Br from plasma.

Precision

The % standard deviation per determination is as low as 6% for analysis of homogeneous samples and 15% for heterogeneous samples. Table 8-1 shows some typical results (14,16). Precision of replicate hair analysis has been reported (4) to be in the range of 0.8 to 14.3% RSD for a series of metals.

Accuracy

Accuracy has been tested by studying results from an interlaboratory comparison (14,16). The majority of the analyses fall within 10% of the given values. A summary of these results are presented in Table 8-2.

Simplicity, Cost, and Sample Throughput

The technique is simple and permits a large sample throughput. The cost, however, is very high since it involves the use of a Van de Graff accelerator and vacuum equipment. Commercial laboratories generally charge $50/sample if no sample preparation is required. There is no commercially available equipment, no computer software or spectral library.

SAMPLE PREPARATION METHODS

Low Temperature Ashing

Principle of the Technique--

See Sections 4 and 7.

Biological Matrices--

The biological matrices tested by low temperature ashing included fat (22), tissues (10,11,17), hair (1,9,16), serum (11,12,15,21), urine (11), and skin (13).

Chemicals Analyzed--

A large number of metals have been analyzed in the literature, including cadmium, lead, zinc, calcium, titanium, manganese, nickel, and strontium (16). Beryllium cannot be analyzed due to its volatility.

TABLE 8-1. RESULTS OF ANALYSIS OF THIN HOMOGENEOUS STANDARD SAMPLES

Element (Compound)	Amount Given μg	PIXE Results Found μg	Number of Determinations	% Standard Deviation Per Determination
Cl (NaCl)	6.8	5.6	3	5.2
Cl (KCl)	6.3	6.6	7	6.7
K (KCl)	7.0	7.1	7	6.5
Ca (CaF)	3.7	3.6	5	9.8
Ti metal	10.2	8.9	3	9.0
Cr metal	7.2	7.1	5	1.0
Fe metal	11.5	13.7	5	3.1
Cu (CuS)	7.9	8.1	14	7.1
Ga (GaP)	4.6	4.6	2	7.8
Br (CsBr)	3.1	3.0	3	3.0
Cs (CsBr)	5.2	5.5	3	6.0

TABLE 8-2. RESULTS FROM AN INTERLABORATORY COMPARISON STUDY

	Sample on Millipore Filter		Sample on Whatman 41 Filter	
	Amount Given ng[a]	Amount Found[a] / Amount Given	Amount Given ng[a]	Amount Found[b] / Amount Given
K	2040	1.032	943	0.981
V	374	1.071	233	1.069
Mn	1649	1.022	693	0.915
Fe	1916	1.093	1269	0.988
Cu	300	1.110	202	1.151
As	356	1.154	244	1.120
Pb	813	1.064	558	1.083
Mean		1.078		1.044

[a] Given amount of each element within area of proton beam striking sample.

[b] Ratio of PIXE result to given amount for 2 to 4 replicate determinations. The standard deviation for a single determination by PIXE is approximately 15%.

Precision and Accuracy--

No data was available. The reported precision and accuracy was not separated from the performance of the analytical technique.

Method Interference--

No data was available.

Recovery Data/Quantitation--No data was available.

Limit of Detection--

No data was available. The reported limit of detection was not separated from the LOD of the analytical technique.

Simplicity, Cost, and Sample Throughput--

See discussion in Section 4.

Chelation/Extraction Technique

Principle of the Technique--

See Section 4.

Biological Matrices--

The biological matrices reported include blood and plasma (7).

Chemicals Analyzed--

Chromium (VI) has been reported as analyzed by the chelation/extraction technique (7).

Precision and Accuracy--

The recovery of chromium (VI) spikes by this method was reported to be >80% at pH 2.4 (7). The total recovery, however, varied from 40 to 90% owing to the difficulty of collecting and depositing the final few microliter on the target foil.

Method Interference--

See Section 4.

Recovery Data/Quantitation--

See Section 4.

Simplicity, Cost and Sample Throughput--

See Section 4.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETER

The PIXE technique is ideally suited for the simultaneous determination of multiple elements in matrices which are not easily digested. The method is expensive and not readily available.

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SECTION 9

SPECTROPHOTOMETRY (COLORIMETRY)

INSTRUMENTATION

Principle of the Technique

Spectrophotometry is the analytical technique which utilizes selective absorption of light by molecules for quantitative and semi-qualitative analysis. The absorption of light represents a quantum mechanical transition between the ground electronic state and an excited state of the molecule. Since this difference in energy is characteristic of a particular molecule, knowledge of the energy (or wavelength) of light being absorbed gives qualitative information about the analyte. While spectrophotometric measurements are a useful source of qualitative information, the selective absorption of light is rarely sufficient for the qualitative analysis of a given sample. Therefore, supporting evidence from alternative analytical method(s) is required for analyte identification. The major application of spectrophotometry is quantitative analysis. The number of photons being absorbed is directly proportional to the number of molecules undergoing the transition, therefore, measurement of the amount of light absorbed gives quantitative information about the analyte.

The general term for chemical analysis using measurement of the absorption of light is absorptiometry, and the term spectrophotometry refers to the use of a spectrophotometer for absorptiometric measurements. The term colorimetry applies to the use of light within the visible wavelength region. When UV energy light is utilized, the technique is called UV absorption spectrometry.

A spectrophotometer consists of basically four parts: a source of photons, usually a tungsten-iodine (320 nm to 3500 nm) or hydrogen (160 nm to 360 nm) lamp; some type of monochromator to spatially separate light of different wavelengths; a cell to hold the sample; and a detector (usually a photomultiplier tube) along with the necessary electronics to measure light energy. Spectrophotometers are divided into two types, single beam and double beam. Most laboratories utilize double beam instruments since they minimize experimental errors which occur during spectrophotometric analysis by measuring power losses from the cell (reflection and refraction), and losses from absorbances of the solvent and any other compounds present at identical concentrations in both cells, thus generating an instrumental blank and automatically subtracting this value from the sample.

Chemicals Analyzed

Spectrophotometry is a useful analytical technique for any compound or complex which absorbs light within the UV-visible energy window. If the analyte of interest does not absorb light within this region, it may be possible to prepare a derivative of the analyte which does absorb, thus, spectrophotometry is applicable to a wide range of analytes, including most organic compounds, and metals via measurement of organometallic complexes. Chemicals cited which have been analyzed by spectrophotometry include lead (1-16), mercury (17,18), zinc (19,20), arsenic (21-28), phenols (30,31), methanol (32) trichloroacetic acid (33,34), trichloroethanol (35), phthalate esters (37), cholinesterase (38), dimethylamine (39) and propranolol in human plasma (40), methylformamide in human urine (41). Infrared spectroscopy was used to determine copran in extracted solutions of chloroform (42).

Methods of Quantitation

The quantitative treatment of spectrophotometric data utilizes Beer's Law. The simplest statement of Beer's Law is:

$$A = abC = \log \frac{P_o}{P}$$

where

A = absorbance

a = absorptivity

b = cell path length

C = concentration, in grams per liter

P_o = level of light power entering cell

P = level of light power leaving cell

P/P_o = transmittance = T

Absorptivity is an intensive property of a given substance, while absorbance is an extensive property and, thus, will vary with the concentration of the sample and the dimensions of the cell used to contain the sample. A convenient measure of absorptivity is the molar absorptivity (E), which is also referred to as the molar (or molecular) extinction coefficient and is defined as:

$$E = \frac{AM}{bC}$$

where M is the molecular weight of the absorbing species. The magnitude of the molar extinction coefficient will determine the limit of detection for a given absorbing species under standard spectrophotometric conditions, and it will relate the absorbance to the number of molecules undergoing electronic transition.

Limit of Detection

The spectrophotometric limit of detection of a given analyte is directly dependent upon its molar extinction coefficient, and the magnitude of these coefficients vary widely. The average spectrophotometric limit of detection is approximately 1 μg (36). Specifically, propranolol has been determined in the concentration level of ng/mL and monomethylformamide in the level of μg/mL.

Precision

The precision of a spectrophotometric determination of a xenobiotic or its metabolite from a biological matrix will be determined by two separate factors: the precision of the spectrophotometric measurement; and the precision of the sample preparation method. Precision evaluations of spectrophotometric measurements typically give a relative standard deviation of two to three percent (36). When sample preparation procedures are included in the relative standard deviation expression, reported values range from 15% (38), 14% (31) to 0.3%, (8) with an average of 7.6% (8,20,26,31,32).

Accuracy

The accuracy of spectrophotometric analysis of xenobiotics and/or their metabolites in biological matrices can be evaluated via measurements of the percent recovery of analytes spiked into the biological matrix. Results of these experiments reported in the literature include:

(1) Pb from blood - 99.8% ± 0.3% (8);
(2) Phenol from urine - 95% ± 7% (31);
(3) Zn from lysed red blood cells - 96% ± 3% (20); and
(4) Trichloroethanol from trichloroethanol-glucuronide complexes from blood - 97.6% ± 5.8% (35).

These experiments give an average recovery of 97% ± 2%.

Simplicity, Cost, and Sample Throughput

A spectrophotometric measurement is a basically simple and quick procedure, requiring only a few seconds for the actual measurement. This technique can be automated, thus, a large number of samples can be analyzed quickly spectrophotometrically. The time-intensive step will be preparation of the sample solution from the biological matrix. Since the absorption of a particular wavelength of light is not unique for a given compound, care must be taken that no other compounds which absorb light of similar energy as that absorbed by the analyte of interest are present in the solution to be spectrophotometrically analyzed. This contrasts with the situation occurring in atomic absorption (AA) and/or atomic emission (AE) spectrophotometry, which have essentially replaced UV-visible absorption spectrophotometry as analytical techniques for the analysis of metals. In AA and AE, the wavelength of light absorbed or emitted is essentially unique to a given metal, thus minimizing interferences. Also, the limit of detection is much lower for AA and AE than for UV-visible absorption spectrophotometry. Chromatography, both gas and liquid, has become the method of choice for the analysis of organic compounds due to the higher degree of selectivity and lower limit of detection. However, spectrophotometry is a well established technique for which instrumentation is widely available.

SAMPLE PREPARATION METHOD

Sample preparation methods for organic compounds are similar to those used for other analytical techniques such as GC and are discussed in detail in Section 11.

Dithizone Spectrophotometry

Principle of the Technique--

Dithizone (diphenylthiocarbazone) is a chelating reagent for many of the transition metals. In this technique, the biological matrix is either wet

(9,12) or dry (12,14,21) ashed and the residue dissolved in acid. The solution is then made basic and dithizone in $CHCl_3$ added to form the colored complex which is spectrophotometrically analyzed. A related chelating reagent is dimethyldithiocarbamate used specifically for Cu (39).

Biological Matrices--

The dithizone-spectrophotometric method has been applied to a large number of biological matrices including blood (2,8-13), urine (3,6,7,9,13,39), skin (12,14), adipose (12,14), and hair (19). It has also been used for the analysis of bone, blood vessels, liver, lymph nodes, kidney, pancreas, spleen, lung, adrenal glands, prostate gland, thyroid gland, testes, brain, stomach, bladder, and heart (14).

Chemicals Analyzed--

Dithizone-spectrophotometry has been used for the analysis of Pb (2,3,5-14), Zn (32), Hg (18), Cd (29) and Cu (39) from biological matrices.

Method Interferences--

The wet or dry ashing removes most interferences except other metal ions which may complex with dithizone. However, since different metal-dithizone complexes absorb different energies of light, these interferences are minimized by discrimination via the spectrophotometer. Interferences from other metal ions can be further minimized by careful pH control of the solution. The most serious interference to lead analysis by the dithizone method is bismuth. Methods to remove this interference have been developed (8).

Concentration Factor - LOD--

The concentration factor necessary for a given biological matrix is directly proportional to the concentration of the analyte in the matrix. For blood analysis, 2-10 mL is typically used for lead analysis. The limit of detection of Pb in blood has been reported as 10 ppb (13).

Recovery Data - Quantitation Method--

The Beer's Law equation is used for quantitation in the dithizone-spectrophotometric analysis using a standard calibration curve.

Precision and Accuracy--

The precision of the dithizone-spectrophotometric analysis has been reported to be ± 0.5 μg Pb lead in blood (2) and ± 0.2 μg Pb over the range of 0.0 to 20.0 μg in standard solutions (8).

Simplicity, Cost, and Sample Throughput--

Analysis of lead, zinc, mercury, and cadmium by the dithizone-spectrophotometric method is both simple and relatively low cost. The rate determining step in the sample throughput is in the ashing step. Wet ashing with acids will take from ~10 minutes with blood and urine to several hours for adipose tissue. Dry ashing requires overnight heating at ~500°C in a muffle furnace.

The simplest dithizone method is the single tube monocolor technique which is used for the determination of lead in blood and urine (15,16).

Silver Diethyldithiocarbamate Spectrophotometry

Principle of the Technique--

Silver diethyldithiocarbamate is a chelating reagent for arsenic. The procedure is similar to that described for dithizone in that the biological matrix is dry or wet ashed. The residue is dissolved in acid and the resulting solution is reacted with potassium iodide, stannous chloride, and zinc to produce arsine. This is then mixed with a solution of silver diethyldithiocarbamate to form a colored complex whose absorption at 525 nm is measured spectrophotometrically.

Biological Matrices--

The silver diethyldithiocarbamate- spectrophotometric method has been applied to several biological matrices including blood (23,28), hair (22-25, 28), urine (22,23,25,26), and skin (28). It has also been used to analyze As in lung, heart, pancreas, spleen, kidney, and small intestine (21), as well as brain and liver tissue (21,28).

Chemicals Analyzed--

This method has only been reported to be used for the analysis of arsenic.

Method Interferences--

The only interference discussed in the literature is sulfide, which can be successfully removed using glass wool impregnated with lead acetate (26).

Concentration Factor - LOD--

The LOD for arsenic is reported to vary from 0.5 μg (26) to 1.0 μg (22,23). For urine analysis typically a 200 mL sample is required, while 3 grams of hair are usually used (22,23).

Recovery Data - Quantitation Method--

The Beer's Law equation is used for quantitation using a standard calibration curve.

Precision and Accuracy--

The precision of the silver diethyldithiocarbamate spectrophotometric analysis is reported to be ±10% with 1.0 μg As sample (26).

Simplicity, Cost, and Sample Throughput--

This procedure is very similar to the dithizone method in simplicity, cost, and sample throughput. The method involves generation of arsine, so special precautions should be taken to minimize laboratory workers exposure.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

In the reported literature, two basic methods were utilized for the spectrophotometric analysis of metals: dithizone and silver diethyldithiocarbamate. The silver diethyldithiocarbamate method was used only for the analysis of arsenic and no discussion of its suitability for any other analytes was found. In theory, the dithizone method is applicable to the analysis of most transition metals. In the cited literature, it was only reported to be used for the analysis of lead (3,9-20), zinc (21), mercury (22), and cadmium (23). No reference to physical parameter of an analyte which would make it suitable for a dithizone spectrophotometric analysis was found.

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SECTION 10

GAS CHROMATOGRAPHY

INSTRUMENTATION

Principle of the Technique

Gas chromatography is a separation technique wherein components of a sample are separated by differential distribution between a gaseous mobile phase and a solid or liquid stationary phase held in a glass or metal column. A sample is injected into the carrier gas as a sharp plug and individual components are detected as they elute from the column at characteristic "retention times" after injection. Many basic texts are available for a detailed description of GC principles and applications.

Instrumentation--

There are several basic components of a gas chromatograph. These include a cylinder of carrier gas with a flow controller and pressure regulator, an injection port for sample inlet, the chromatographic column, the detector, and a recorder system.

In order to avoid excessive, injector related band broadening, the sample should be introduced onto the column instantaneously as a plug. Gaseous samples are usually introduced using syringes or bypass sample loops. Reproducibility is better than 0.5% relative standard deviation with sample loops. The standard technique for injecting liquids is to introduce a syringe needle through a self-sealing septum and inject measured volumes from an attached syringe. These syringes are commercially available at various volumes down to one-tenth microliter and with proper handling can give a reproducibility of ±2% relative standard deviation. For packed column chromatography, sample sizes ranging from 1-5 µL can be introduced onto the column without reducing column resolution or efficiency. Specialized injection techniques have been developed for capillary column chromatography in order to split the injected sample and produce a small (0.001-0.1 µL) sample for column injection. Capillary columns have smaller diameters (0.25-0.5 mm) than packed columns (2-4 mm) and lower carrier gas flow rates (1-3 mL/min for capillary, 10-40 mL/min for packed). This dictates that the injection volume for capillary columns be much smaller than that used with packed columns in order to achieve the high separations capillary columns offer.

The chromatographic column is the heart of any gas chromatographic system. Both packed and capillary columns are used for analysis depending upon specific separation requirements.

Packed columns usually consist of a solid support coated with a liquid phase packed into a short (approximately 180 cm) ~2 mm I.D. column. These columns are relatively easy to work with, inexpensive to make, and available in many liquid phase/solid support combinations which can be tailored towards a given analysis. Because of these advantages and their high loading capacity, which allows the separation of relatively large masses of solutes, packed columns are routinely used for simple analyses. However, they do possess several inherent disadvantages. Columns with a high loading of liquid phase tend to bleed (i.e., elute some of the stationary phase or its decomposition products) and, therefore, a number of good gas chromatographic liquid phases (DEGS, FFAP, etc.) are incompatible with selective GC detectors. Most importantly, packed columns provide inherently low resolution which often gives incomplete separations of complex mixtures.

Capillary columns provide an alternative to packed column chromatography. In wall coated open tubular columns (WCOT), the liquid phase is coated directly onto the surface of the capillary tube (~0.2 mm I.D.). Thin films (~.2 µm) and small I.D.s provide for increased chromatographic resolution resulting from rapid mass transfer. Capillary columns can be prepared at significantly greater lengths than packed columns, since the pressure drops associated with the packed columns are no longer present. Although capillary columns have always provided high separation efficiency, problems with adsorption and degradation of sample components, limited availability of chromatographic phases, rapid column deterioration, and problems with column and sample handling limited their general use. However, recent developments in column technology, coating procedures, and injection techniques have resulted in the use of capillary columns as the primary technique for routine analysis in state-of-the-art laboratories.

The chromatographic column separates the components present in an injected sample. These components must be measured by a detector as they elute from the column. Desirable characteristics of a detector are high sensitivity, low noise level, wide linearity of response, responses to compounds of interest, ruggedness, insensitivity to flow and temperature changes, and low cost. There is no ideal detector for all substances, but good detectors have been developed. The thermal conductivity cell and flame ionization detector are the most universal and widely used detectors. Specific detectors such as the electron capture detector and nitrogen/phosphorous detector have the advantage that they selectively detect only certain chemical types. This makes them extremely useful for trace and qualitative analysis.

Differentiating detectors are the commonly used GC detectors because of their convenience and accuracy. The response of these detectors is proportional to the concentration or mass flow of the eluted component. The thermal conductivity detector is the most familiar example of a detector responding to concentration. The flame ionization detector responds to mass flow rate. The chromatogram produced by a differentiating detector consists of a series of peaks for each component. The area under each peak is proportional to the total injected mass of that component. This assumes a constant flow rate during the analysis when measuring with a concentration-sensitive detector like the thermal conductivity detector.

Since chromatographic detectors differ greatly in operation, it is difficult to compare them; however, certain characteristics indicate the usefulness of a detector, including selectivity, sensitivity, response, noise, minimum detectable quantity, and linear range. There are secondary characteristics which should also be considered. If possible, the detector should be simple, inexpensive, rugged and insensitive to changes in carrier gas flow rate and temperature. Tables 10-1 and 10-2 summarize detector parameters for the most common gas

TABLE 10-1. SUMMARY OF DETECTOR PARAMETERS, I[a]

Detector	Principle of Operation	Selectivity	Sensitivity	Response (coul/gm)
Thermal Conductivity	Measures thermal conductivity of gases	Universal - responds to all compounds	6×10^{-10} g	-
Gas Density Balance	Difference of molecular weight of gases	Universal - responds to all compounds whose MW differs from carrier gas	Variable in range of T.C.D. (g)	-
Flame Ionization	H_2-O_2 Flame 2000°C Plasma	Responds to organic compounds not fixed gases or water	9×10^{-13} g/sec for Alkanes	0.01
Electron Capture	N_2 (carrier) + $\beta \rightarrow e^-$	Response to electron adsorbing compounds. esp, halogens, nitrates and conjugated carbonyls.	2×10^{-14} g for CCl_4	-
H^3 Ni^{63}	e^- + sample → loss of e^- from plasma		5×10^{-14} g for CCl_4	-
Flame Photometric P compd.	Alkali modified H_2-O_2 1600°C plasma	enhanced response to phosphorus compounds	4×10^{-14} g/sec	-
N compd.		enhanced response to nitrogen compounds	7×10^{-12} g/sec	-
Helium	He + $\beta \rightarrow$ He* He* + Sample → $(\text{Sample})^+$ He + e^-	Universal - responds to all compounds	2×10^{-14} g for methane	28.
Cross Section	β + sample → e^- + $(\text{Sample})^+$	Universal - responds to all compounds	10^{-9} g	-

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[a] Data, in part, from H.M. McNair and E.J. Bonelli, Basic Gas Chromatography, Varian Instrument Division, Palo Alto, CA, 1968.

TABLE 10-2. SUMMARY OF DETECTOR PARAMETERS, II[a]

Detector	Linear Range	Minimum Detectable Quantity (gm)	Stability	Temp. Limit °C	Carrier Gas	Remarks
Thermal Conductivity	10^4	10^{-5} gm of CH_4 per ml of detector effluent	good	450°C	He H_2 N_2	non destructive - requires good temp and flow control. Simple, inexpensive and rugged.
Gas Density Balance	10^3		good	Better sensitivity 150°C	N_2 CO_2 Ar	good for analysis of corrosive compounds. non-destructive
Flame Ionization	10^7	2×10^{-11} gm for Alkanes	excellent	400°C	He N_2	$H_2O + CS_2$ good solvents because no response. destructive
	5×10^2	10^{-13} gm for lindane	fair	225°C	N_2 or	Detector is easily contaminated and easy to clean. Sensitive to water; carrier gas must be dry. Can be operated in pulsed or D.C. mode. non-destructive
Electron Capture	50	4×10^{-12} gm for lindane	fair	350°C	Ar + 10% CH_4	
H^3 Ni^{63}	10^3	2×10^{-12} gm parathion	fair	300°C	N_2	Destructive - requires flow controller for hydrogen and air
Flame Photometric P compd.	10^3	2×10^{-10} gm azobenzene	fair	300°C	He	Destructive - requires flow controller for hydrogen and air. high sensitivity operating in starved O_2 mode.
N compd.	5×10^3	10^{-12} gm for fixed gases	poor	100°C	He	High sensitivity to bleed precludes its use with columns other than active solids. non-destructive
Helium						
	10^4	10^{-5} gm	good	225°C	H_2 or He + 3% CH_4	
Cross Section						

[a] Data, in part, from H.M. McNair and E.J. Bonelli, Basic Gas Chromatography, Varian Instrument Division, Palo Alto, CA, 1968.

chromatographic detectors. The information in this table includes principles of operation, selectivity, sensitivity, linear range, limit of detection, stability, temperature limit for the detector and common carrier gases used.

Performance Parameters--

The basic equation of chromatographic performance is the Van Deemter equation which can be written in simplified form as:

$$H = A + \frac{B}{u} + Cu$$

where H is the plate height (also HETP or height equivalent to a theoretical plate), A represents eddy diffusion or nonuniformity of flow, B represents the contribution to H by molecular or longitudinal diffusion, C is the resistance to mass transfer term and u is the flow rate of the carrier gas. For the most efficient separation, H should be as small as possible. The eddy diffusion or "A" term is defined as:

$$A = 2 \lambda d_p$$

where d_p is the diameter of the packing particles and λ is a structural constant related to the particular packing material in use. In order to minimize the contribution of the "A" term to H, it is clear that small, uniform particles are desired as the packing. Note that "A" is the only term independent of flow rate. In addition, the "A" term becomes negligible in open-tubular, or capillary, columns. The "B" terms, or molecular diffusion, is defined as:

$$B = 2 D_G \gamma$$

where D_G is the diffusion coefficient of the solute in the gas phase and γ is the tortuosity factor. γ is approximately 0.63 for a packed column and 1 for a capillary column. The contribution of molecular diffusion to the plate height will be most pronounced at low flow rates.

The resistance to mass transfer, or "C" term, arises from the nonequilibrium condition that exists when a solute molecule is partitioning between the stationary phase and the carrier. That is, equilibrium between the mobile and stationary phase for the solute is never achieved. Clearly, this nonequilibrium is worse at higher flow rates. This term is directly proportional to d_p^2 (or r^2 in capillary columns) and d_F^2 (thickness of stationary phase film on the particles or the wall, in the case of capillary columns) clearly indicating that the most efficient columns contain small particles that are lightly loaded, i.e., have a low weight stationary phase/weight packing ratio.

In practical terms, the number of theoretical plates (N) a column can produce is defined as:

$$N = 5.54 \frac{t_R}{W_{1/2}}^{2}$$

where t_R is the retention time of the solute of interest and $W_{1/2}$ is the peak width at half maximum of that solute. The plate height is obtained simply by dividing N by the column length L.

Chromatographic theory suggests several parameters which can be used to improve column efficiency. First, for packed columns, efficiency is improved by the use of small uniform particle sizes. Second, each column has an optimum carrier gas flow rate for maximum efficiency. Third, although the detector usually dictates the choice of carrier gas, a high molecular weight gas (small D_G) should be used for the highest efficiency. This efficiency, however, is achieved only at low flow rates which lengthens the analysis time. Conversely, when rapid analysis is required and highest efficiency is not necessary, a low molecular weight (larger D_G, but also larger optimum flow rate) carrier gas such as helium or hydrogen is preferred. Fourth, low liquid loadings on solid packing (1-10%, small d_F) increase column efficiencies while also allowing faster analysis and lower temperature operation. Low loadings, however, reduce the sample capacity and may require highly inactive solid supports. Fifth, although most GC analyses are performed at an outlet pressure of one atmosphere and flow rate requirements fix the inlet pressure, best efficiency is obtained at low inlet to outlet pressure ratios. This limits how small d_P can get. This aspect is, in part, responsible for the higher efficiencies of capillary columns. Finally, resolution can usually be improved by lowering the column temperature, thus increasing the retention, but also the number of partitionings that can take place. A practical limit to lower temperature is the resulting increase in analysis time. However, long isothermal analysis times increase the contribution to H from the B and C terms and produce broader peaks.

The relative retention (α, "solvent efficiency") is a measure of the selectivity of a particular staionary phase and is derived from the specific interactions of the solute with the stationary phase. It is defined as:

$$\alpha_{2,1} = \frac{t_{R2}-t_N}{t_{R1}-t_N} = \frac{K_2}{K_1} = \frac{k_2}{k_1}$$

where t_{R2} is the retention time of component 2, t_{R1} is the retention time of component 1, t_N is the dead-time (time required for gas to flow through column with no retention), K is the distribution coefficient of the component between gas and liquid phases and k,

$$\frac{t_R-t_N}{t_N},$$

is the capacity ratio. The capacity ratio, k, corrects K for the relative amounts of mobile phase and stationary phase present in a given column. That is, k is dependent on the particular column and K is an inherent description of the solubility of a particular solute in a particular stationary phase. Four interactive forces determine distribution coefficients for a given solute and include orientation or van der Waals forces, induced dipoles, dispersion or London forces, and specific interaction forces resulting from chemical banding or complex formation. The familiar McReynold's numbers can be used to assist in the choice of a stationary liquid phase which will have a selectivity for the compounds of interest. Numerous selective liquid phases are available which facilitate separation of a wide variety of sample components.

Chemicals Determined

Gas chromatography, as a technique, is applicable to any chemical which can be volatilized in the GC injection port and is not thermally labile. Compounds most easily determined by gas chromatography are those with boiling points lower than 300°C and that are relatively nonpolar. Polar compounds such as alcohols, acids, and amines, tend to adsorb on column surfaces to give poor chromatographic peak shape and higher detection limits. If these polar compounds are to be analyzed by gas chromatography they are often derivatized to minimize solute:sorbent interaction and peak tailing, thus improving compound separation.

As a general guideline, Table 10-3 lists specific chemical classes and the various liquid phases which have been used for the analysis in human biological matrices. Information in this table include analyte type, liquid phase, and column type, along with the specific citation. Of note in the table is the large number of different column packings used for packed column analysis. The table contains relatively few references for capillary columns; however, it is apparent that their many advantages indicate that they will become the predominantly used column type in the future. The markedly higher separating power of capillary columns, together with recent improvements in the inertness and ruggedness of the columns themselves provides a versatility that is unmatched in packed columns. This versatility enables the researcher to analyze virtually the entire range of organic compound types with only two or three different liquid phases, thereby reducing both cost of analysis and analysis time.

Method of Quantitations

For quantitative analysis by gas chromatography, the detector signal must be related to a given concentration or total amount of analyte injected on column. Detector response can be measured either as a chromatographic peak height or a chromatographic peak area. These measurements can be determined manually or by integrator response. The internal (1,2,3) standard or the external standard (4) technique are the most common methods for quantitating by GC analysis. For the external standard technique, calibration solutions of the components of interest are prepared in the same solvent as the sample components. These calibration solutions are injected onto the GC and absolute detector response is measured for known concentrations of the analytes of interest. Concentration in an unknown sample is then determined by taking the ratio of the detector response for the sample against the detector response for the standard. For the internal standard technique, a calibration standard is prepared which contains an internal standard at a known concentration. The same internal standard is also added to the unknown sample. Using this method of quantitation, peak heights are ratioed to the detector response for the internal standard and the concentration of the analyte in the sample is determined by the ratio of these relative peak heights. Other techniques, such as the method of standard addition, have been used but with only limited application. The use of the internal standard technique relies upon the proper selection of an internal standard which will not coelute with any sample components, is soluble in the standard solvent, shows similar chromatographic behavior as the test components, and gives a suitable detector response. The use of an internal standard minimizes errors due to uncertainties in final sample volume and injection volume.

Quantitation of a single component analyte is a straightforward calculation which simply relates area response of the sample to component concentration in the standard. However, quantitation of multicomponent analytes such as polychlorinated biphenyls (PCB), polychlorinated terphenyls (PCT) and polychlorinated naphthalenes are more difficult. Since the chromatographic detector responds differently to each isomer in a mixture, a direct comparison of total peak areas between standard and sample will give spurious results if individual peaks are obscured by interfering material, missing or have different intensities (5). Several approaches have been taken to circumvent this problem. Methods used for quantitation of PCBs will be used to illustrate these methods, but the methods are also applicable to other multicomponent analytes. As a first approach, PCBs in samples have been chlorinated to form decachlorobiphenyls. For quantitation, the chromatographic signal for this derivatized sample is compared to the chromatographic response of a derivatized standard containing a known amount of Aroclor 1254. This approach gives a total PCB value and has also been applied to PCTs (6). In many cases, this approach is undesirable because an extra analytical step is required and because any evidence of metabolism or degradation of the sample is destroyed. As an alternative, Webb and McCall (7) proposed a quantitation method for electron capture detection based on individual peak comparisons between the sample peaks and peaks of identical retention times from Aroclor reference materials in which each individual Aroclor peak is a known weight percentage of total. Weight percentages of the Aroclors were determined by using common GC separations and halogen specific detection for determining the chlorine

TABLE 10-3. SELECTED PHASES FOR ORGANIC ANALYSIS

Compound Class	Examples	Liquid Phase	Column Type	Reference
Acids				
Derivatized	2,4-D	SE-30	P[a]	15
		DEGS	P	16
	Dicamba, 2,4-D	SE-30/OV-210	P	17
		OV-17	P	18
	Fatty acids	OV-17	SCOT[b]	19
	Oxalate	DEGA	P	20
	Oxalate	Unisole F200	P	21
Underivatized	CN^- as HCN	Porapak Q[c]	P	22
	Fatty acids	Chromosorb 10 1[c]	P	23
	Fatty acids	SP-1200/H_3PO_4	P	24,25
	Fatty acids	SP-1000/H_3PO_4	P	25
Phosphoric Acids				
Derivatized		OV-210	P	26
		SE-30/QF-1	P	26
Alcohols				
	Methanol	Chromosorb 102[c]	P	1
		Carbowax	P	27
		Carbowax 20M	C	28
Aldehydes				
	Acetaldehyde	PEG	P	29
	Acetaldehyde	Porapak Q[c]	P	30
	Acetaldehyde	Tenax GS[c]	P	31
Amides				
	Dimethylformamide	Carbowax	P	32
	Acetamide	SP-1000/H_3PO_4	P	33

(continued)

TABLE 10-3. (continued)

Compound Class	Examples	Liquid Phase	Column Type	Reference
Amines				
	3-Aminobiphenyl	Dexsil 300	P	34
Carbamates		Carbowax 20M	P	35
Esters	Phthalates	DEGS	P	36
	Phthalates	SE-30/OV-210	P	8
Ethers	Dieldrin	Apiezon L/QF-1	P	37
	Polychlorinated Dibenzofuran	SE-30	P	9,38
		OV-225	P	39
Volatile Halogenated Aliphatics				
		OV-17/OV-210	P	39
		Molecular sieve^c	P	40
	1,1,1-Trichloroethylene	Carbowax 20M	P	32,41
		SP-1000/Carbowax 1500	P	42
	$CHCl_3$	n-Octane	P	43
	$CHCl_3$	SE-52	C^d	44
Halogenated Hydrocarbons				
	PCBs	OV-1	P	2,6,11,45
	Lindane	DOW-111	P	46
	DDT	OV-210	P	4,47-49
	DDT	DC-200/QF-1	P	2,10,50-52
		QF-1	P	50,53
	DDT, DDE	SE-30/QF-1	P	3,47
	DDT, DDE	OV-17/QF-1	P	47,54,55
	Heptachlor epoxide	OV-17/OV-210	P	38,47,56-59
	PCBs	SE-30/OV-210	P	8,4,12,49,57
	PCBs	SE-54	C	14
	PCBs	Apiezon M	C	14
	PCBs	SP-2100	C	14
	PCBs	C-87	C	14
	PCBs	SE-52	C	14

(continued)

TABLE 10-3. (continued)

Compound Class	Examples	Liquid Phase	Column Type	Reference
Halogenated Hydrocarbons (continued)				
	DDE, PCBs	OV-17	P	45,48,60
	BHC	AC-QF-1	P	61
	Dieldrin, PBBs	OV-101	P	49,62-64
	PCBs	OV-3	P	10
	Polychlorinated Tephenyls	Dexsil 300	P	6
	PCBs	SE-30	P	5,13,65
	PCB, DDT	QF-1/SF-6	P	66,67
	OC Pesticides, PCBs	OV-225	P	58
Hydrocarbons	Alkylbenzenes	Halcomid	P	68
	Styrene	Carbowax 400	SCOT[b]	69
Nitrosamines	N-Nitrosodimethylamine	Carbowax 20M	P	70,71
Phenols	Chlorophenols	DEGS	P	72
	Chlorophenols	Butane-1,4-diolsuccinate	P	72
		Tenax GC[c]	P	73
	PCP	SE-30	P	74,75
	PCP	OV-17	P	76
	p-Nitrophenol	DF-1	P	77,78
	p-Nitrophenol	OV-1	P	78
	Chlorophenols	OV-210	P	78,79
		DC-200	P	78
	Naphthol	OV-17/QF-1	P	75,77,80

(continued)

TABLE 10-3. (concluded)

Compound Class	Examples	Liquid Phase	Column Type	Reference
Metals[e]	Hg	Activated carbon[c]	P	81

[a] Packed column.

[b] Support coated open tubular column.

[c] Solid support - no liquid phase.

[d] Capillary column.

[e] Although not an organic compound, mercury present in hair and liver was separated, in the vapor phase, from interfering pyrolysis products and detected using atomic absorption spectroscopy. This report is included due to the novel use of vapor/solid adsorption. The author sees no reason why volatile organometallics (e.g., tetraethyl lead) cannot be analyzed by gas chromatography as well. No articles to this effect were found in the review of the literature, probably because of the biological matrix limitation.

content, and mass spectrometry for determining the average molecular weight for each peak. Other approaches to quantitating PCBs have included using area responses for selected peaks (6,8-12) or peak heights relative to a one PCB peak (13). Unfortunately, none of these approaches are very accurate or precise for PCB quantitation. With recent developments in high resolution chromatography and electron capture detectors, separation of all 209 PCB isomers can be achieved during GC analysis with capillary columns using temperature programming (14). Under these conditions, it is possible to calibrate an instrument with individual PCB isomers which then allows quantitation of the individual sample components. A total PCB concentration can be calculated by summing values for the individual isomers. This method offers the advantages of improved accuracy and precision as well as the ability to identify and quantitate individual isomers in a sample.

A principal component pattern recognition technique (SIMCA) (82) has been applied to the problem of establishing similarities among, and differences between, sample residue profiles. In this approach, the data from selected peaks are expressed as fractional parts of the total. From these data, a mathematical model is generated for a group of samples. Relations among samples in this group can then be viewed in two dimensional plots of principal component projects from the higher dimension data space. The SIMCA technique has been applied to PCB residue profiles in sediment, water and aquatic biota (83), but not to in-human biological matrices.

Limits of Detection

The limits of detection for any gas chromatographic technique are dependent mainly on the detector systems summarized in Table 10-1. However, for some compounds that do not chromatograph well, i.e., those which show reversible or irreversible adsorption on the column's surface, this adsorption behavior rather than detector response will determine the limits of detection for that chromatographic system.

Precision and Accuracy

As stated previously, the precision for any injection technique should be ±2% expressed as relative standard deviation.

Simplicity, Cost, and Sample Throughput

Gas chromatography is generally a simple technique for the analysis of organic compounds. Improvements in instrumentation, GC columns, column packings, detectors, and automated systems for both sample injection and data processing have all contributed to the ease of operation for GC techniques. Gas chromatographs are moderately expensive pieces of equipment ranging in cost from $10,000 for very basic instruments to $50,000 for instruments equipped with multiple detection systems, pneumatics, and automation equipment. Sample throughput is variable depending upon the compounds for analysis and the sample matrix. Generally, analysis times by packed column chromatography range from 30 to 45 minutes. Analysis time by capillary column chromatography can be somewhat longer and can require analysis times as long as three hours for very difficult separations if optimum resolution is required, such as for PCB isomer determinations.

SAMPLE PREPARATION METHODS

See Section 11.

RECOMMENDED METHOD ANALYTE CORRELATION PARAMETERS

Two requirements must be met if a compound is to be analyzed by gas chromatographic methods: it must be sufficiently volatile to pass through a gas chromatograph and it must be stable at the temperature required for volatilization. Generally, compounds with boiling points less than 300°C or which can be derivatized to a compound with a boiling point less than 300°C are considered sufficiently volatile for GC analysis. Because of the wide variety of specialized packing materials, most compounds, regardless of functional group substituents, can be analyzed by gas chromatography. Polar compounds are often derivatized to achieve better chromatographic behavior. See Table 10-2 in this section for more detailed information.

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SECTION 11

PREPARATION METHODS FOR ORGANIC ANALYSIS

Preparation methods for organic analysis usually involve methods for extracting, concentrating, and fractionating the analyte from the sample matrix and other chemicals in the sample. The methods are generally applicable to the determination of organics by a range of analysis methods including gas chromatography, high pressure liquid chromatography, thin layer chromatography, or mass spectrometry. For simplicity, all organic preparation techniques applicable to these methods will be discussed within this section.

EXTRACTION TECHNIQUES

Static Headspace Analysis

Principle of the Technique--

Static headspace analysis is a method for determining volatile compounds in liquids or solids by measuring vapor phase components which are in thermodynamic equilibrium with the sample of interest in a closed system. For aqueous systems, the distribution of sample components between water and gaseous phases depends upon their water solubility and vapor pressure at the equilibration temperature. For example, compounds with a high vapor pressure and low solubility will preferentially partition into the vapor phase.

Headspace analysis is most often used in conjunction with gas chromatography and will be considered exclusively in this sense. For quantitative analysis, chromatographic peak area (F_i) is the parameter measured and at relatively low pressures is proportional to the partial vapor pressure (P_i) of the volatile component i in the headspace.

$$F_i \propto P_i \qquad \text{(I)}$$

$$F_i = \bar{C}_i P_i \qquad \text{(II)}$$

where $\bar{C}_i$ is a constant dependent upon the chromatographic detector. The partial vapor pressure P_i is generally expressed as

$$P_i = P_{oi}X_i\gamma_i \qquad \text{(III)}$$

where P_{oi} is the vapor pressure of i, X_i is the mole fraction of i, and γ_i is the activity coefficient of component i dissolved in the sample. Substituting equation (III) into equation (II) shows the relationship between peak area (F_i) and the above parameters.

$$F_i = C_iP_{oi}X_i\gamma_i \qquad \text{(IV)}$$

For a given sample, with a fixed chromatographic system (X_i and C_i are constant), peak area and, hence, sensitivity may be varied by changing either P_{oi} or γ_i. Vapor pressure (P_{oi}) may be increased by increasing the equilibration temperature, whereas the activity coefficient is most effectively increased by adding electrolyte to the solution, i.e., "salting out."

In static headspace analysis, equilibrium is achieved using a sealed glass container at a constant temperature. Equilibration time depends upon the sample volume and equilibration temperature and, in most cases, even with large samples, does not exceed 60 minutes.

After equilibration, a volume of headspace is withdrawn and injected onto the chromatographic column using a gas tight syringe or a gas sampling loop. Since the sample is a gas, a heated injection port is unnecessary making this technique well suited for heat labile compounds. Usually, packed column gas chromatography has been used for analysis; however, adaptation to high resolution capillary columns with no loss in quantitative precision and a significant improvement in retention time accuracy and chromatographic separation has been reported.

Biological Matrices--

The static headspace technique has been used for the analysis of organic compounds in blood (1-10), urine (11,12,13,15), and adipose samples (1). For example, the analysis of methylene chloride in blood and urine was performed by equilibrating a 10 mL sample in a 25 mL Erlenmeyer flask at ~75°C for 5 minutes, then directly purging the headspace in the flask onto a GC column for a 60 second period. Methanol in urine was analyzed by equilibrating 1 mL partial vapor pressure P_i is generally expressed as

$$P_i = P_{oi} X_i \gamma_i \qquad \text{(III)}$$

where P_{oi} is the vapor pressure of i, X_i is the mole fraction of i, and γ_i is the activity coefficient of component i dissolved in the sample. Substituting equation (III) into equation (II) shows the relationship between peak area (F_i) and the above parameters.

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Chemicals Analyzed--

Any compound which will give a vapor pressure over the sample matrix can be analyzed by static headspace analysis. Obviously, the higher the vapor pressure, the more suitable the technique. Low molecular weight, low boiling point, hydrophobic compounds are best suited to this technique.

It was reported that vapor phase partitioning for the following chemical classes followed the order: alkanes > olefins > cycloalkanes > aromatics. Within each chemical class, an increased vapor partition was observed as the molecular weight increased. A study of a series of n-alkane standards showed that even C_{22} could be detected, although its boiling point is greater than 300°C. With salting-out and sufficiently high equilibration temperatures, water soluble compounds including methanol, ethanol, acetone, and methyl ethyl ketone are also amenable to analysis. However, use of elevated temperatures during equilibration may prevent the analysis of heat-sensitive compounds.

References reported the analysis of xylene (2,13), ethyl benzene (12), dichloromethane (2,16), 1,1,1-trichloroethane (14), dichlorofluoromethane (1), trichloroethylene (4), trichlorofluoromethane (1), cyanide (7), toluene (5), acetaldehyde (3,6,8-10), ethanol (17) and methanol (13) in various biological matrices.

Method Interferences--

Endogenous material in urine has been reported to interfere with the analysis of methanol in urine using GC/FID (13). The determination of acetaldehyde in blood is often hindered by the conversion of the acetaldehyde to ethanol, or vice-versa, during workup. These interferences can be minimized by adding chloral hydrate (6), sodium nitrite with sulfosalicylic acid (9) or sodium azide (10) to the sample before workup or by conversion of the aldehyde to the semicarbazone prior to the removal of the red blood cells (3). The aldehyde is reformed during precipitation of the serum proteins with trichloroacetic acid.

Concentration Factors/Limit of Detection--

Detection limits for static headspace analysis depend upon compound type, GC detector, conditions for equilibration, and volume of sample injected. A detection limit of 100 ppb of methanol in urine has been reported using GC/FID (13). Detection limits for more hydrophobic compounds, or compounds which give a more sensitive detector response could give significantly lower detection limits. A limit of detection of 20 ppb was reported for CH_2Cl_2 using flame ionization detection (2).

Recovery Data/Quantitation Method--

Since static headspace analysis depends upon partitioning and high recoveries are not generally expected, recovery data are not usually given. However, a recovery of 50 ± 11% was reported for CH_2Cl_2 in blood (2) and recoveries of 100% ± 4% RSD (3) and 90% ± 3.8% RSD (6) were reported for acetaldehyde in blood. A 76% ± 3.4% RSD recovery for the determination of cyanide as HCN in blood has been reported (7).

Accurate quantitative analysis depends upon the calibration procedure. Since the partition coefficient of the solute in the equilibrated gas sample system is a function of its activity coefficient, calibration solutions should closely approximate the sample matrix. Quantitation can be performed by response factors generated from a calibration curve. The accuracy of this method depends upon how closely the standards resemble the sample matrix. As an alternative, quantitation can be performed using the method of standards addition.

Method Precision--

Quantitative headspace analysis can be a very precise procedure. However, good reproducibility depends upon two factors, namely, exact temperature control during equilibration, and a reproducible method for transporting and injecting the headspace sample into the gas chromatograph. Relative standard deviations of less than 5% have been reported for the analysis of methylene chloride in blood and urine (2), methanol in urine (13), cyanide in blood (7), as well as acetaldehyde in blood (3,6,10).

Simplicity, Cost, and Sample Throughput--

Static headspace analysis is a simple, rapid method for analyzing volatile organics in biological samples. Several commercial instruments are available for automated headspace analysis to further reduce processing costs. The rate limiting step for the entire procedure is usually the gas chromatographic analysis. Depending upon GC conditions, 2 to 3 samples can be analyzed per hour.

Recommended Method/Analyte Correlation Parameter--

As stated previously, any compound which will give a vapor pressure over the sample matrix can be analyzed by static headspace analysis, and the higher the vapor pressure the more suitable the technique. The two physical properties which determine vapor pressure are boiling point and solubility in the sample matrix. Boiling point remains constant regardless of sample matrix and those compounds with the lowest boiling points are most suitable for headspace analyses. Solubility will depend upon the sample matrix; for blood and urine which are predominantly aqueous samples, hydrophobic or nonpolar compounds will partition into the headspace most easily. Conversely, for adipose samples, lipophobic or polar compounds are best suited to this technique. To summarize this information, boiling points and water:octanol partition coefficients (as a measure of polarity) could be used as method/ analyte correlation parameters.

Dynamic Headspace Analysis

A widely used method for isolating volatile organic materials (boiling point <200°C) from liquids is purging the sample with prepurified gas, collecting the stripped materials on a sorbent trap, and analyzing the trapped compounds by thermal desorption followed by gas chromatography or gas chromatography/mass spectrometry. Alternatively, for samples which tend to foam, the sample headspace may be purged instead of the sample itself.

Removal of organic compounds by sparging with an inert gas is frequently referred to as volatile organic analysis (VOA) or purge and trap. The technique depends upon partitioning of the compounds between the liquid and gaseous phases. This partitioning is a function of sample temperature, gas-liquid interfacial area, and the solubilities (<2%), volatilities (<200°C), and aqueous activity coefficients of the test compounds. In addition, the partition rate depends on the flow rate and total volume of the purge gas.

Modifications of the above described static headspace methodology have been described in at least two analyses (18,19). The first describes the analysis of styrene in adipose by passing nitrogen over a small amount of heated adipose tissue and collecting this gas which would contain the volatilized compound (15). The entire volume of gas collected was subsequently injected into the GC. Although it is expected that by constantly exposing the sample to "fresh" extracting gas, a more complete recovery would be achieved based on a more favorable vapor-phase equilibrium in the extraction system, this more efficient extraction may compromise the chromatography by requiring extremely large gas injections. This paper (18), unfortunately, gave no results indicative of recovery, precision, etc.

The assembled purge apparatus consists of a container with a purge gas inlet, a device for regulating sample temperature, and a purge gas outlet through a sorbent cartridge (20,22).

The cartridge is composed of material with a high affinity for organic compounds, and preferably, a low affinity for the liquid sample. Tenax GC is the most commonly used trapping material. It is thermally stable, contains few background contaminants, provides few sites for irreversible adsorption, is chemically inert, and has a low affinity for water. Its major drawback is its low affinity for a number of very volatile organic compounds including the lower alkanes and methanol (23). Alternate sorbents have also been employed. EPA Method 624 for the analysis of volatile organics currently uses a silica gel/Tenax trap (24).

Sample volumes vary from 0.5 to 1000 mL depending upon the type of sample and concentration of the substances to be determined. Gas flow rates range from 20 to 200 mL/min and purge times from a few minutes to several hours depending, again, upon the sample concentration and the appropriate breakthrough volume for the target analyte on the sorbent cartridge.

There are a number of considerations involved in determining purge temperature. Even though maximum stripping occurs at 90 to 98°C, these elevated temperatures may cause problems with condensation on the trap material, artifact formation, and thermal decomposition. Furthermore, samples which tend to foam do so to a greater degree at higher temperatures. For these reasons, analysis of low molecular weight, hydrophobic compounds is performed at ambient temperatures. Compounds with boiling points less than 200°C and aqueous solubilities less than 2% are amenable to this type of analysis. Elevated temperature purge and trap techniques have been reported for a number of volatile polar organic compounds (25-28). Recoveries of many of these compounds improved significantly with the addition of salt to the aqueous solution prior to analysis (26).

Analytes trapped on the sorbent cartridge are introduced into the chromatographic system using thermal desorption. At this point test components may be either focused in a cryogenic trap (28) or transferred directly to the GC column (24). For packed column analysis the latter approach is generally used. The purge and trap technique can be performed with the cartridge directly on-line to the gas chromatograph and detector system.

For the separation of volatile organics, both capillary (28) and packed (24) columns have been used. Chromosorb 101, Carbowax 1500 and 20M, SE-30 and OV-101 are the most frequently used liquid phases for GC analysis. Fused silica capillary columns with bonded phases have become popular for all GC analysis including the analysis of volatile compounds. However, the use of an on-line purge and trap system interfaced to a capillary column causes several difficult problems. During capillary column chromatography the sample components must be introduced onto the column as a sharp band to avoid deteriorating chromatographic resolution. This may be achieved by cryotrapping using either a cryofucusing unit (28) or by cooling a small portion of the capillary column to liquid nitrogen temperatures (28). Unfortunately, the volume of water collected on the trapping material is often large enough to cause freezing during the focusing procedures. The use of a water cooled condenser or a condenser tube to remove water from the gas stream prior to the sorbent cartridge has been reported (29). A dry purge of the sample cartridge to remove water after the load operation has also been reported (28,30). Other researchers have split the gas stream after desorption to minimize the amount of water entering the chromatographic system (31). However, this significantly decreases method sensitivity.

Biological Matrices--

The purge and trap technique has been used for the analysis of organic compounds in blood (22,25,32), blood serum (20,23), urine (24), and adipose tissue (23). Blood and blood plasma are purged with or without aqueous dilution, and often require the use of an antifoaming agent (23). Urine is purged without additional treatment (24). Purging of adipose tissue was accomplished by pushing strips of tissue to the bottom of a prepurged, purge vessel, and immersing the lower portion in a 115°C glycerol bath (23).

Chemicals Analyzed--

Most organic compounds with boiling points less than 200°C can be successfully purged from water. Successful purging of these compounds from biological matrices is less certain due to the increased complexity of the sample matrix. Compounds which have been reported to successfully purge include acetone, dichloromethane, chloroform, benzene, carbon tetrachloride, pentan-2-one, ethyl benzene, xylene, styrene, trimethyl benzene, dichlorobenzene, and dimethyl ethyl benzene from blood (22,25). Chloroform, bromodichloromethane, dibromochloromethane, bromoform, carbon tetrachloride and 1,2-dichloroethane were reported by purge and trap analysis from both blood serum and adipose tissue (23). Trace levels of organotin and organosulfur compounds were purged from urine (24). These and other volatile halocarbons were reported by purge and trap analysis of blood (32).

Method Interferences--

No data available.

Concentration Factors/Limit of Detection

Detection limits for purge and trap analysis depend on sample size, GC detector and compound type. Table 11-1 shows reported limits of detection for many halogenated and nonhalogenated purgeable compounds (32).

Recovery Data/Quantitation Method

Table 11-1 lists recovery data for the purge and trap analysis of blood for a number of volatile compounds (32). Recoveries vary from compound to compound, but are generally good. Peoples et al. (23) report essentially quantitative recovery of chloroform, carbon tetrachloride, trichloroethane, bromodichloromethane, dichloroethane, dibromochloromethane and bromoform from both blood serum and adipose tissue.

Method Precision

The precision of purge and trap analysis is closely related to the complexity of the sample matrix. Table 11-1 lists standard deviations obtained via purge and trap analysis of blood.

Simplicity, Cost, and Sample Throughput--

Analysis of volatile compounds in biological matrices by purge and trap analysis is a relatively simple technique. The laboratory equipment required beyond the gas chromatograph is fairly inexpensive. Depending on GC conditions, 2 to 3 samples can be analyzed per hour.

Recommended Method/Analyte Correlation Parameters--

As stated previously, purge and trap analysis is directly related to both the boiling point and the solubility of the analyte of interest in the sample matrix.

Solvent Extraction

Principle of the Technique--

Extraction of organic compounds from biological matrices occurs when the solvent is brought into contact with the sample. If the analyte of interest has a higher affinity for the extracting solvent than the sample matrix, then it will partition into the solvent. The percent of analyte extracted into the solvent phase (%E) can be calculated by

$$\%E = \frac{100\ K_D}{K_D + \frac{W_s}{W_o}}$$

TABLE 11-1. INSTRUMENTAL LIMITS OF DETECTION AND RECOVERIES OF VOLATILE ORGANIC COMPOUNDS IN BLOOD ANALYZED BY PURGE AND TRAP

Compound	LOD[a] (ng)	% Recovery ± S.D.[b]
Chloroform	4.1	92 ± 48
1,1,1-Trichloroethane	5.5	115 ± 101
Carbon tetrachloride	1.2	29 ± 13
Trichloroethylene	1.2	149 ± 65
Tetrachloroethylene	2.9	93 ± 29
1,2-Dichloroethane	9.0	88 ± 50
Vinylidine chloride	4.2	102 ± 27
Bromodichloromethane	16.3	105 ± 34
Dibromochloromethane	9.6	102 ± 60
Dibromochloropropane	12.1	48 ± 18
Bromoform	13.8	126 ± 48
Chlorobenzene	1.1	91 ± 56
m-Dichlorobenzene	2.9	68 ± 11
p-Dichlorobenzene	2.7	91 ± 11
o-Dichlorobenzene	2.7	105 ± 15
Benzene	3.4	82 ± 103
Styrene	3.7	91 ± 15
Ethyl benzene	6.4	93 ± 36
m-, *p*-Xylene	7.2	100 ± 42
o-Xylene	8.7	NT[c]

[a] Limit of detection, signal to noise ratio = 4.

[b] % recovery = mean of 5 replicates ± standard deviation.

[c] Not tested.

where K_D is the distribution coefficient of an analyte between the solvent or solvent mixture and the matrix and W_s and W_o are weights of the sample and solvent, respectively. From this equation, it is obvious that high analyte recoveries will depend upon either a large distribution coefficient or a small sample:solvent ratio.

The choice of solvent is critical to liquid-liquid extraction (LLE) procedures. As discussed above, the solvent must have a large distribution coefficient for the compounds of interest. Extensive listings of partition coefficients from water are available for various solvent systems. Partition coefficients for all organic compounds are higher for neutral species than for compounds with an electrostatic charge.

Along with having a high extraction efficiency for the analytes of interest, the extracting solvent should be immiscible with the sample, not contain contaminants which might compromise subsequent analysis, be chemically inert, be specific for the compounds of interest, and be amenable to the analytical method of choice. Since solvent evaporation may be employed to further concentrate sample extracts, solvents with low boiling points are preferred.

Solvent selection is particularly important for analysis of halogenated organics by electron capture detection. Solvents such as methylene chloride or chloroform cannot be used due to a high detector response for these solvents. In addition, since EC detection is so sensitive, i.e., ~1 pg, solvent purity is critical.

Pentane (35), hexane (36-68,102,103), benzene (69-73,104,105), acetonitrile (86,87), carbon disulfide (88), and petroleum ether (89-94) have been used for the extraction of relatively nonpolar compounds such as PNAs, PCBs and organochlorine pesticides or the extraction of polar compounds which have first been derivatized to reduce polarity. A variety of solvent mixtures including methylene chloride:methanol (78,79), benzene:acetone (80), hexane:acetone (34,76), and hexane:isopropanol (77) have also been used to extract organochlorine pesticides from various biological matrices. Solvents such as diethyl ether (60,81-83), benzene (84), ethyl acetate (85), and methylene chloride (96-98) or solvent mixtures such as toluene:ethyl acetate (95) have been used to extract the more polar organics.

The addition of salt to the aqueous matrix will increase the activity coefficients of organic analytes to effectively increase the partition coefficient into the organic solvent. Sodium sulfate (96) is commonly used to achieve salting-out. Unfortunately, the addition of salt increases the density of aqueous solutions which may promote emulsion formation when heavier-than-water solvents are used for extraction.

The extraction of acids and bases first requires that the analyte be electrostatically neutral. For this reason, acids are extracted at a low pH (47,60,69,82-87,99). Conversely, bases are extracted at an alkaline pH.

In vivo binding of organic chemicals to proteins in biological samples may reduce recoveries during solvent extraction (39). The addition of formic (39,41,43,45,66) or sulfuric acid (60,75) to plasma appears to release the bound organics. Urine can be hydrolyzed with hydrochloric acid (85), sulfuric acid (33) or sodium hydroxide to release conjugated phenols.

Operationally, there are several ways in which solvents may be physically brought into contact with the biological sample. These include mechanical shaking or stirring, Soxhlet extraction, refluxing, and homogenization.

Mechanical shaking is the most common method for extracting blood and urine samples (35,39-41,43-45,49,52-55,57,61,67-71,87,75,83-85,92,95-99,104-106). During extraction the sample is simply placed in a container with the extracting solvent and mixed for a fixed period of time ranging from several minutes to several hours. Phase separations are achieved by centrifugation for samples which tend to form emulsions. Simple mechanical mixing has also been reported for the extraction of organics from fat (55,88,90,80) with phase separations effected by low temperature precipitation (80,90).

Homogenization is the most common method for extracting adipose samples (23,47,48,50,56,76,78,79,91,100,106) or other tissues such as liver (94). The sample is cut into small pieces, mixed with an inert material such as sea sand or sodium sulfate, and homogenized with solvent over a short period of time. Separation of the sample and solvent is accomplished by centrifugation or filtration.

Soxhlet extraction is the final method reported for solvent extraction of adipose samples (86). During extraction, the sample is placed in a thimble in the Soxhlet tube, solvent evaporates from a boiling flask, condenses, and passes through the sample. The sample extract then returns to the solvent pot. This evaporation, condensation cycle is repeated many times during the extraction procedure. Soxhlet extraction has the dual advantage of leaving the sample cool and providing multiple extractions over an extended period of time. To improve sample:solvent contact and dry the sample, adipose may be ground with anhydrous sodium sulfate prior to extraction.

Biological Matrices--

As discussed above, solvent extraction techniques have been used to isolate organic chemicals from blood, urine, and adipose sample. The technique has also been applied to the determination of PCBs and DDT in hair samples (101) as well as spleen, liver, kidney and other tissues (63,94). Mother's milk has also been analyzed for PCBs (52) and pesticides such as Lindane and DDT (61,68).

Chemicals Analyzed--

Solvent extraction methods are most routinely applied to semivolatile or nonvolatile organic compounds. Since some solvent evaporation is usually required to concentrate the sample, compounds with boiling points less than 150°C would experience volatility losses. When solvent concentration is not used, low molecular weight halogenated hydrocarbons in urine have been analyzed by GC/ECD using pentane as the extracting solvent (35). Analogously, styrene in fat has been analyzed by GC/FID using carbon disulfide as the extracting solvent (55). Since these volatile compounds may elute with the extracting solvent during GC analysis, the success of the procedures depends upon selectivity of the GC detector.

Nonpolar compounds will efficiently partition into most organic solvents. Polar compounds can be extracted with acceptable recoveries if more polar solvents and larger solvent to sample ratios are used. The latter is a feasible approach, since most biological samples are small (i.e., 2 to 20 mL) and increasing the solvent sample ratio still does not require a large volume of extracting solvent. Phenols and carboxylic acids are extracted from blood and urine after addition of a mineral acid to lower the pH and form the nonionic species (33,47,82-84,95,96,99).

Method Interference--

Interferences may result from three sources, namely, organics of biological origin which are extracted from the sample, anthropogenic chemicals which are extracted from the sample, and interferences which result from dirty glassware, solvent, etc. The first two types of interferences are usually removed using sample fractionation and cleanup procedures or selective gas chromatographic conditions (these methods are discussed in detail in Section 10). It is essential that good quality control procedures are used to identify and remove interferences caused by sample contamination.

Concentration Factors/Limit of Detection--

Most solvent extraction methods for isolating organic compounds from biological matrices use small sample sizes ranging from 1 (39,41,43,45,49,53,96) to 10 (35) mL for blood and urine, and 0.2 (55) to 10 (77,91) g for adipose tissue. If the extracted organics are concentrated into 1 mL of solvent, concentration factors between 0.2 and 10 are achieved. However, larger samples may be used [i.e., 180 g of fat (48)] to give correspondingly greater concentration factors.

As with any method, limits of detection will depend upon compound type, GC detector, concentration factor, and fraction of the final extract submitted for analysis. Table 11-2 summarizes reported detection limits using a variety of solvent extraction techniques.

Recovery Data--

Table 11-3 shows the applicability of the solvent extraction techniques to the analysis of organic compounds in biological matrices. In this table, recoveries for various classes of compounds are given as a function of biological matrix and extracting solvent. Most reported recoveries were calculated for spiked vs. unspiked sample matrices. However, Dale et al. (40) were able to demonstrate that in vivo binding of DDT to blood proteins reduced recoveries during hexane extraction. The addition of formic or sulfuric acid appears to release the bound pesticide to give quantitative recoveries.

TABLE 11-2. REPORTED DETECTION LIMITS FOR SOLVENT EXRACTION TECHNIQUES

Compound	Detection Limit (ppb)	Matrix	GC Detector	Reference
Phenols				
phenol	100	Urine	FID[a]	107
dichlorophenol	1000	Urine	FID	107
trichlorophenol	1000	Urine	FID	107
2,4-dichlorophenol	4690	Adipose	ECD[b]	141
2,3,5-trichlorophenol	100	Blood	ECD	141
2,3,5-trichlorophenol	240	Adipose	ECD	141
2,4-dichlorophenol	780	Blood	ECD	141
2,4,5-trichlorophenol	320	Adipose	ECD	141
2,4,6-trichlorophenol	90	Adipose	ECD	141
2,3,4,5-tetrachlorophenol	190	Adipose	ECD	141
2,3,4,6-tetrachlorophenol	90	Adipose	ECD	141
pentachlorophenol	140		ECD	141
2,4,5-trichlorophenol	70	Blood	ECD	141
2,4,6-trichlorophenol	30	Blood	ECD	141
2,3,4,5-tetrachlorophenol	80	Blood	ECD	141
2,3,4,6-tetrachlorophenol	40	Blood	ECD	141
pentachlorophenol	30	Blood	ECD	141
pentachlorophenol	5	Adipose	ECD	47
p-nitrophenol	25	Urine	ECD	99
1-naphthol	20	Urine	ECD	88
hexachlorophene	10	Blood	ECD	108
Esters				
di-n-butylphthalate	10	Urine	ECD	109
diethylhexylphthalate	5			
Halogenated Hydrocarbons				
$CHCl_3$	1	Serum,Urine	ECD	93
DDE	1	Blood	ECD	56
	2	Blood	ECD	75
	1	Blood, Mother's Milk	ECD	32
	1	Blood	ECD	57
	1	Blood	ECD	42
	800	Adipose	ECD	56
DDD	1	Mother's Milk, Blood	ECD	32
DDT	3	Blood	ECD	56
	3	Blood	ECD	42
	1	Blood, Mother's Milk	ECD	32
	2	Blood	ECD	44
	2	Blood	ECD	57
	2	Blood	ECD	75
	100	Fat	ECD	54
	400	Fat	ECD	56

(continued)

TABLE 11-2 (continued)

Compound	Detection Limit (ppb)	Matrix	GC Detector	Reference
Lindane	0.3	Blood	ECD	42
hexachlorobenzene	1	Blood	ECD	109
hexachlorobutadiene	1	Blood	ECD	109
Dieldrin	1	Blood	ECD	57
β-BHC	1	Blood	ECD	57
organochlorine pesticides	100-300	Adipose	ECD	110
	10	Adipose	ECD	54
	1	Blood	ECD	75
Chlordane	10	Blood	ECD	75
Dicofol	10	Blood	ECD	75
Torpent	10	Blood	ECD	75
Captan	10	Blood	ECD	75
polychlorinated naphthalenes	100	Blood	ECD	75
PCB	100	Blood	ECD	75
	100	Adipose	ECD	54
Benzene derivatives	50	Adipose	FID	88
Nitrogen-Containing Components				
Dioxacarb	205	Urine	AFID[c]	95
Carbofuran	210	Urine	AFID	95
OMS-22	190	Urine	AFID	95
Several aromatic amines (e.g., benzidine) as pentafluoropropionyl derivatives)	1000	Urine	ECD	96

[a] Flame ionization detector.

[b] Electron capture detector.

[c] Alkali flame ionization detector.

TABLE 11-3. REPORTED RECOVERIES FOR SOLVENT EXTRACTION TECHNIQUES

Compound	Recovery (%)	Matrix	Reference
Acids			
2,4-D	95	Blood	83
	93-107	Blood	69
	93-107	Urine	69
Propanoic	93	Urine	98
Phosphates			
DMDTP[a]	10	Urine	112
	47 ± 10	Urine	98
	81 ± 8	Urine	98
DEDTP[b]	10	Urine	112
	98 ± 9	Urine	98
	93 ± 6	Urine	98
DMP[c]	10-30	Urine	112
DED[d]	55-70	Urine	112
DETP[e]	44	Urine	112
Phenols			
cresol	100	Urine	107
dichlorophenol	93	Urine	107
pentachlorophenol	85-98	Adipose	19
p-nitrophenol	92-100	Urine	99
2,4-dichlorophenol	101	Adipose	141
2,3,5-trichlorophenol	91	Adipose	141
2,4,5-trichlorophenol	97	Adipose	141
2,4,6-trichlorophenol	85	Adipose	141
2,3,4,5-tetrachlorophenol	90	Adipose	141
2,3,4,6-tetrachlorophenol	84	Adipose	141
pentachlorophenol	91	Adipose	141
2,4-dichlorophenol	63	Blood	141
2,3,5-trichlorophenol	76	Blood	141
2,4,5-trichlorophenol	77	Blood	141
2,4,6-trichlorophenol	89	Blood	141
2,3,4,5-tetrachlorophenol	81	Blood	141
2,3,4,6-tetrachlorophenol	W2	Blood	141
pentachlorophenol	99	Blood	141
naphthol	85-95	Urine	88
hexachlorophene	90-100	Blood	108
Halogenated Hydrocarbons			
DDE	90	Blood	44
	92	Adipose	113
	80-99	Adipose	114
	58	Blood	52

(continued)

TABLE 11-3 (continued)

Compound	Recovery (%)	Matrix	Reference
	58	Blood	40
	98	Blood	42
	82	Blood	86
	>80	Adipose	90
	78	Blood	86
	100	Blood	42
	45	Blood	40
	80-97	Adipose	113
	83	Adipose	114
	90	Blood	44
hexachlorobenzene	92	Blood	110
	86-99	Adipose	91
hexachlorobutadiene	92	Blood	110
heptachloroepoxide	100	Blood	41
	82	Blood	40
Dieldrin	78-99	Adipose	99
	91	Blood	40
	98	Blood	42
Lindane	82	Blood	53
	79	Blood	43
	99.8	Serum	105
α-BHC	75	Blood	40
γ-BHC	85	Blood	40
Δ-BHC	76	Blood	40
Aldrin	29	Blood	40
β-BHC	93	Blood	42
organochlorine pesticides	84	Blood	43
	85	Adipose	92
PCB	85-95	Adipose	114
	80-100	Adipose	54
	87-109	Adipose	100
PBB	90-110	Adipose	109
	90 ± 2	Blood	36
light halogenated hydrocarbons	90-96	Urine	36
Benzene Derivatives			
styrene	97 ± 4	Adipose	87

[a] 0,0-dimethyl phosphorodithioate

[b] 0,0-diethyl phosphorodithioate

[c] dimethyl phosphate

[d] diethyl phosphate

[e] diethyl phosphorothioate

Precision--

Precision of solvent extraction methods is good for most compounds. Percent relative standard deviations (RSD) are generally less than 10%. Table 11-4 lists reported RSD values for a range of organic compounds.

Simplicity, Cost, and Sample Throughput--

Solvent extraction is a simple, rapid method which does not require specialized equipment. Without including subsequent cleanup steps, it should be possible to process 10 to 15 samples per day using solvent extraction techniques.

Recommended Method/Analyte Correlation Parameters--

Volatility and polarity are the two physical properties which will most significantly effect analyte recovery using solvent extraction procedures. If a solvent concentration step is required during sample work up, the boiling point of the analyte should be at least 100°C greater than the solvent. Generally, a water:octanol partition coefficient greater than 5 is required to achieve good recovery during extraction. Compounds with partition coefficients less than 5 can be extracted if a large solvent/sample ratio is used. Acids and bases should be in their undissociated form to achieve highest partition coefficients. Finally, the analyte must be inert to the conditions of the extraction, i.e., acid, base, solvent, or solvent contaminants, and oxygen.

Solvent Dilution

Principle of the Technique--

During solvent dilution the sample is simply dissolved in an organic solvent. Once dissolved, the sample cleanup or fractionation and GC analysis is performed.

Biological Matrices/Chemicals Analyzed--

In order to use solvent dilution, both the sample matrix and analytes must be soluble in the solvent. This technique has been applied to the analysis of hexachlorobenzene, organochlorine pesticides, and PCBs in adipose samples (115,116).

Concentration Factor - LOD--

No concentration is achieved using this method.

TABLE 11-4. REPORTED PERCENT RELATIVE STANDARD DEVIATIONS (RSD) FOR SOLVENT EXTRACTION TECHNIQUES

Compound	%RSD	Matrix	Reference
Phenols			
p-nitrophenol	2[a]	Urine	99
	14[b]	Urine	112
cresol	1-8[a]	Urine	107
dichlorophenol	4[a]	Urine	107
hexachlorophene	15-20[a]	Blood	108
Phosphates			
DMDTP[c]	20[b]	Urine	98
	10[b]	Urine	98
DEDTP[d]	10[b]	Urine	98
	6[b]	Urine	98
Halogenated Hydrocarbons			
BHC isomers	6[b]	Blood	104
DDE	3[a]	Blood	39
	6[a]	Blood	41
	5[b]	Blood	57
DDT	4[a]	Blood	39
	6[b]	Blood	104
	10[b]	Blood	57
dieldrin	15[b]	Blood	57
HCB	3-10[a]	Blood	91
PBB	2[b]	Blood	36
Benzene Derivatives			
styrene	4[b]	Adipose	87

[a] Calculated from replicate samples.

[b] Calculated from replicate spikes.

[c] O,O-dimethyl phosphorodithioate

[d] O,O-diethyl phosphorodithioate

Recovery Data--

Although recovery from a solvent dilution technique should be quantitative, losses could occur during subsequent cleanup or fractionation steps. Quantitative recoveries were reported for hexachlorobenzene spiked into chicken fat (101). Recoveries included a Florisil cleanup procedure.

Precision--

No data are available, but the absence of analytical preparative steps should result in low systematic errors.

Simplicity, Cost, and Sample Throughput--

Sample dilution without cleanup is extremely simple and rapid. However, this technique usually requires a cleanup step prior to analysis which makes the final procedure more time consuming.

Recommended Method/Analyte Correlation Parameters--

Solubility in the proposed solvent would be the best parameter to predict method performance.

Sorbent Columns

Principle of the Technique--

The use of sorbent columns in the determination of organic compounds is an application of liquid-solid chromatographic techniques (LSC). Compounds are isolated from the sample matrix by adsorption onto a solid phase which is composed of small particles of commercially available materials including carbons, resins, and foams. The removal of the compounds of interest is accomplished by washing the solid phase with a solvent or solvent mixture for which these compounds have a high affinity. The partitioning of the analyte species thus takes place between the solid and the eluting liquid. In practice, the solid phase particles are packed in a column, and the liquid sample is passed through the column at a slow rate. The column is rinsed with a small volume of the appropriate solvent and the eluting material is collected and concentrated for analysis.

Biological Matrices--

Sorbent column cleanup and/or concentration is particularly useful for the analysis of aqueous-like matrices, such as urine (117-119).

Chemicals Determined--

Sorbent column cleanup has been used for sample cleanup and concentration during the analysis of acetamide (117) and various chlorinated and nonchlorinated phenols (118,119).

Method Interferences

No data available.

Recovery Data

Recoveries greater than 80% were reported for the analysis of chlorinated phenols in urine using XAD-2 resin to preconcentrate the phenols after hydrolysis (119). Based on this information, recovery from the sorbent cleanup step alone should be good.

Precision--

No data available.

Simplicity, Cost, and Sample Throughput

Since sorbent columns require only simple laboratory equipment, small quantities of relatively inexpensive reagents, and minimal amounts of labor, they result in high sample throughput and low cost.

Recommended Method/Analyte Correlation Parameter

Insufficient data available.

CLEANUP/FRACTIONATION METHODS

Steam Distillation

Principle of the Technique--

Steam distillation is a method for separating chemicals on the basis of vapor pressure differences. At a given temperature, compounds with vapor pressures higher than their environment will volatilize and may be collected by cooling. As a sample cleanup technique for biological matrices, water is added to a sample, then the water and volatile portion of the sample are allowed to distill, condense, and are collected. Nonvolatile sample components such as salts, lipids, and proteins remain in the pot.

Biological Matrices--

This method has been applied to urine (14,116,119), blood samples (119,120), and tissue homogenates (119) prior to solvent extraction and GC analysis.

Chemicals Determined--

Steam distillation can be used to fractionate any compound with a vapor pressure equal to or lower than water or which forms an azeotrope with water. The method has been applied to the analysis of phenol, chlorophenol, 2,4- and 2,6-dichlorophenol, 2,4,6-trichlorophenol, and 2,3,5,6-tetrachlorophenol in urine (107) and volatile nitrosamines in blood (119,120) or urine (14), as well as tissue (119).

Method Interferences--

4-Heptanone was identified as an early eluting peak in chromatograms of all urine samples.

Recovery Data--

Recovery data for the analysis of phenols in urine ranged from 90 to 102% for phenol through 2,3,4,6-tetrachlorophenol (107). Recoveries of nitrosodimethylamine (NDMA), nitrosodiethylamine (NDEA), and nitrosopyrrolidine (NPYR) in concentrations of 40, 80, and 90 ng added to 20 mL of blood were 44, 43, and 58%, respectively (120). Recoveries of NDMA, NDEA, N-nitrosodi-N-propylamine (NDPA), N-nitrosodi-N-butylamine (NDBA), N-nitrosopiperidine (NPIP), NPYR, and N-nitrosomorpholine (NMOR) spiked into calf tissue were 72, 84, 87, 66, 83, 62 and 54%, respectively (119). Reported recoveries are for all steps of the analytical methods including distillation, solvent extraction, and solvent concentration.

Precision--

The precision of the analytical method for phenols in urine was tested by analyzing a urine specimen, spiked at 10 mg/L, fifteen times. The relative standard deviation (RSD) of compounds ranged from 1.8% for cresol to 4.2% for 2,4-dichlorophenol. The precision of the method (119) for nitrosamines ranged from 7% RSD for NDMA to 26% RSD for NMOR in tissue and from 5% RSD for NDPA and NPIP to 15% RSD for NMOR in urine.

Simplicity, Cost, and Sample Throughput--

Steam distillation requires specialized equipment for sample processing. Depending on the sample volume, distillation times can range from 30 minutes to several hours. Under these conditions, sample throughput would be low unless a large number of distillation apparatus are available.

Recommended Method/Analyte Correlation Parameters--

Boiling point is the best parameter for predicting method performance. Compounds must have boiling points lower than water or they must azeotrope with water. In addition, compounds must have an affinity for water so they will dissolve in water during condensation. Very volatile or hydrophobic compounds would be lost during condensation.

Vacuum Distillation

Principle of the Technique--

Vacuum distillation operates in the same manner as steam distillation just described except that no water is added to the sample. The system is operated under reduced pressure in order to increase the vapor pressure of the compounds of interest without using excessively high temperatures.

Biological Matrices--

This method has been applied to the analysis of blood samples (121,122).

Chemicals Determined--

Vacuum distillation has been applied to the isolation of short-chained volatile fatty acids.

Method Interferences--

2-Methylbutyric acid was noted to coelute with isovaleric acid in one method (122).

Concentration Factor/Limit of Detection--

A concentration factor of 40 with an LOD of 0.1 μ mol/L is noted in one method (121) and an LOD of 0.1-0.3 μ mol/L for C_3-C_8 with 1 μ mol/L for C_2 in the other (122). These limits, of course, could be decreased if more than 1 mL of serum is used.

Recovery Data--

Recoveries of 95 to 102% for acetic to hexanoic acid, 85% for acetic, and 65% for the octanoic acids were reported (122) in one case and 90 to 110% (acetic to hexanoic) in the other (121).

Precision--

The precision of the analytical methods incorporating vacuum distillation were 3 to 14% RSD (122) and 8 to 19% RSD (121).

Simplicity, Cost, and Sample Throughput--

Although simple in concept, this method requires specialized equipment and can be time consuming as in the case of steam distillation.

Recommended Method/Analyte Correlation Parameters--

Again, boiling point is the best parameter for predicting method performance. Due to the necessarily large amounts of analyte required, this method is not recommended when the analysis of low levels of, e.g., acids, is desired.

Saponification

Principle of the Technique--

Saponification involves the hydrolysis of fats in the presence of base to form ethers and the salts of carboxylic acids. During saponification of biological samples, the sample or the sample extract is refluxed in the presence of base in alcohol [usually 1N KOH in ethanol (48,51,123,124,125)]. Once hydrolysis is complete, the alcoholic solution is extracted with a nonpolar solvent such as hexane. Under these conditions, hydrophobic compounds including pesticides and PCBs are extracted into the nonpolar solvent. The carboxylic acid salts and ethers formed during saponification remain in the alcohol layer.

Biological Matrices--

Saponification is performed on biological samples with a high lipid content, including adipose tissue (37,48,50,51,125), blood (49,88,123,124), and urine (125).

Chemicals Determined--

Saponification has been used for sample cleanup during the analysis of TCDD (125), polychlorinated biphenyls (123,124), polychlorinated dibenzofurans

(48,51), cyclodiene pesticides (34), and polychlorinated terphenyls (123,124). The method can be applied to the analysis of any chemical which is nonpolar and inert to alkaline hydrolysis.

Method Interferences--

PCBs and pesticides can interfere in the determination of TCDD (125), however, this can be minimized by the appropriate use of selective ion monitoring in mass spectroscopy.

Recovery Data--

Tetrachlorodibenzo-p-dioxin was recovered from adipose 40 to 130% (125).

Precision--

For the determination of TCDD in tissue (125), the precision is ±20% for 1 to 1250 pg.

Simplicity, Cost, and Sample Throughput--

Saponification requires special equipment and glassware for refluxing the sample and extracting the saponified mixture. Availablity of clean glassware would usually be the limiting factor for sample throughput. Where sufficient glassware and equipment for heating samples is available, approximately 15 samples could be processed per day.

Recommended Method/Analyte Correlation Parameters--

Compound polarity, as measured by the water:octanol partition coefficient is the best parameter for predicting method performance. Large partition coefficients (>25) are desirable, which would allow the analyte to partition into the hydrophobic solvent while leaving the saponified fat in the alcoholic solution. The analyte must be inert to alkaline hydrolysis.

Acid Hydrolysis

Principle of the Technique--

During this reaction, a carboxylic ester is hydrolyzed to a carboxylic acid and an alcohol in the presence of aqueous acid. For biological samples, acid hydrolysis is used to remove fats from the sample extract. For hydolysis, the extract of the biological material in hexane or a solvent mixture containing hexane is shaken with fuming sulfuric acid (43,55,66,68,74,76,81,126). Hydrolysis with concentrated HCl at 100°C for one hour has been used in the workup of urine for the analysis of chlorinated phenols (119). The fat in the sample is hydrolyzed to a polyhydric alcohol and a carboxylic acid. Under these conditions, hydrophobic compounds remain in the hexane, while the carboxylic acid and alcohol partition into the sulfuric acid phase.

Biological Matrices--

Acid hydrolysis is performed on sample extracts from matrices with a high lipid content including adipose tissue (55,74,76,81), blood (43,66,68), mother's milk (68), and various tissues including liver and skin (126).

Chemicals Determined--

Acid hydrolysis has been used for sample cleanup during the analysis of polychlorinated terphenyls (55,74), polychlorinated biphenyls (74,76,126), DDT (50,52,72), and other organochlorine pesticides (43,80,81,126). The method should be applicable as a cleanup procedure for any compound which is nonpolar and inert to acid hydrolysis.

Method Interferences--

No data available.

Recovery Data--

Recoveries of 84% for organochlorine pesticides from spiked blood were reported using an extraction/hydrolysis procedure. Recoveries greater than 80% were reported for the analysis of chlorinated phenols in urine using XAD-2 resin to preconcentrate the phenols after hydrolysis (118). Based on this information, recovery from the hydrolysis step alone should be good.

Precision--

Precisions of 6% RSD were reported for the extraction/hydrolysis procedure for DDT and BHC insecticides in human blood (66) and 6.2% RSD for the analysis of chlorinated phenols in urine.

Simplicity, Cost, and Sample Throughput--

Acid hydrolysis of biological extracts usually involves only a simple mixing of the extract with acid in a centrifuge tube followed by removal of the organic phase. Not only is this a simple and rapid procedure, it does not require specialized glassware or equipment and therefore allows a very high sample throughput (~20 sample/ h).

Recommended Method/Analyte Correlation Parameter

Compound polarity, as measured by the water:octanol partition coefficient is the best property for predicting method performance. Large partition coefficients (>25) are desirable, which would allow the analyte to partition into the hydrophobic solvent while leaving the saponified fat in the alcoholic solution. The analyte must be inert to alkaline hydrolysis (acid).

Solvent Partition

Principle of the Technique--

Solvent partition processes depend upon the preferential distribution of solute into one of two intermittently contacted but immiscible liquid phases. Solute solubility may be altered by controlling the pH of the aqueous phase, which is the basis for the familiar acid/base wash sequences used to advantage in numerous extraction systems.

In the most common application of solvent partitioning, batch mixing of the biphasic system is accomplished manually with separatory funnels. Although a knowledge of distribution coefficients for specific compounds or classes of compounds can be of practical benefit, the exact procedure (choice of solvents, number of extractions, amounts of solvents, etc.) has most often been determined empirically. The majority of procedures divide samples into polar and nonpolar fractions. During processing, the sample is extracted with a solvent mixture containing both a polar and a nonpolar solvent [i.e., chloroform:methanol (12,78), hexane:formic acid (39,41,45) or hexane:isopropanol (77)], this extract is then added to water and the hydrophobic solvent removed for analysis. As an alternative the sample may be extracted with a polar solvent such as acetonitrile (86,87) or a polar solvent mixture such as ethanol:diethyl ether (84). The extract is then partitioned with hexane to separate the polar from the nonpolar sample components. Partitioning of hexane extracts with acetonitrile (119) or dimethylformamide (127) has also been used for purification of samples.

Biological Matrices--

Solvent partitioning as a cleanup procedure has been applied to extracts of adipose (46,64,77-79,86,127) and blood samples (39,41,45,78,86,87,110,128).

Chemicals Determined--

Hydrophobic compounds are most commonly fractionated using solvent partitioning schemes. The method has been applied to the analysis of organochlorine pesticides (39,41,45,64,127), DDE (41,86,87), DDT (41,86,87), DDE (41,42,77), hexachlorobenzene (77,86,88), heptachloroepoxide (41), Lindane (41), hexachlorobutadiene (110), other organochlorine pesticides (39,41,45,46,78,79,87,110), polychlorinated biphenyls (77), polychlorinated dioxins (128) and polychlorinated benzofurans (128).

Method Interferences--

Without column cleanup procedures, PCBs may interfere with the analysis of organochlorine pesticides. The identity of individual pesticides has been verified using a second GC column or GC/MS analysis (4). An unidentified component in blood interfered with the analysis of hexachlorobenzene and α-BHC using an OV-210/OV-17 column. An SE-30/QF-1 column effectively separated the unidentified compound from the pesticides of interest (110).

Recovery--

Reported method recoveries for DDE (87,127), DDT (87,127), DDD (87,127), hexachlorobenzene (88), hexachlorobutadiene (110), heptachloroepoxide (41), BHC isomers (127), Dieldrin (127), and Aldrin (127) were greater than 80%. Based on this information, recovery from the solvent partition step alone should be good. Low recoveries were reported for hexachlorobenzene using an acetonitrile:petroleum ether partition scheme due to poor partitioning into the petroleum ether (115).

Precision--

A 6% relative standard deviation was reported for the recovery of DDE spiked into 10 blood samples at 10 ppb. Again, this value reflects precision of the entire extraction/fractionation/analysis procedure but indicates precision of the fractionation step alone should be good.

Recommended Method/Analyte Correlation Parameters--

Compound polarity, as measured by the water:octanol partition coefficient is the best property for predicting method performance. Large partition coefficients (>25) are desirable, which would allow the analyte to partition into a hydrophobic solvent from a more polar solvent or water.

Column Chromatography

Principle of the Technique--

Column chromatography has been extensively used to provide the necessary prefractionation of biological samples. This two-phase separation technique is distinguished from other fractionation methods (liquid-liquid partition, thin-layer) by utilization of a stationary phase contained as a packed column bed which is continuously eluted with a liquid mobile phase. The stationary phase may be coated on a solid support or the support itself may function as the stationary phase.

Column chromatography has been employed in two forms, open-column and high performance (HPLC), which differ primarily in the degree of resolution possible and the extent of instrument sophistication required. Open-column chromatography is characterized by the use of stationary phase supports with a particle size range of 75-300 microns. Although the resolution obtained with such large particle supports is inherently low (50-200 theoretical plates/m), the cost of materials and associated equipment is also low. The high permeability of these supports allows adequate flow rates of liquid phase to be achieved utilizing simple gravity-feed techniques in a vertical column arrangement. Separation times using this method are often relatively slow (hours-days); however, this is offset to some degree by its inexpensive nature which allows a number of columns to be set up and run simultaneously.

Open column clean-up systems are used for the separation of one compound or class of compounds from other materials that interfere with analysis. The compounds separated usually possess grossly different properties from the interfering substances, and are thus amenable to low resolution separation. The most common application involves the use of liquid-solid chromatography, wherein a crude sample containing relatively nonpolar analytes is introduced onto a column containing a surface-active support such as silica gel. The column is washed with a non-polar solvent which elutes the analyte(s), but is incapable of eluting more polar compounds. The eluted fraction is thus purified. Changing eluting solvent strength permits the release of more polar species. It is possible by the same process, to free polar analytes from less polar interferences. In this case the nonpolar wash fraction is discarded, and the analyte(s) eluted using stronger solvents.

The process can also utilize other chromatographic modes such as liquid-liquid and gel permeation chromatography.

Biological Matrices--

The use of open column chromatography has been reported for a wide variety of biological matrices. The data base accessed for this task includes reports of column cleanup for blood/serum/plasma (36,42,49,50,52,78,86,112,123,128,132), fat (54,75,77,80,92,101,120,129,130), and urine (111,131). No data were available on column chromatographic cleanup methods applied to extracts of nails or skin.

Chemicals Analyzed--

The vast majority of materials analyzed using open column chromatography are nonpolar, lipophilic compounds such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). Homologous species (polychlorinated terphenyls), isomers (polybrominated biphenyls), and analogs such as polychlorinated dibenzofurans have been separated from, adipose and blood interferences. More polar analytes, such as phthalate esters (128) have been subjected to Florisil column purification.

For the column types most commonly used for cleanup (i.e., silica gel, alumina, Florisil), certain types of compounds are not suitable for separation from matrix components. Polar analytes such as carboxylic acids, amides, sulfonic acids, polyhydric alcohols, certain amines, and other materials are so strongly retained that recovery is adversely affected. Silica gel in particular is subject to significant dissolution in polar mobile phases such as methanol, water, and acetic acid.

Method Interferences--

Most of the reports dealing with column cleanup procedures involved analysis of trace levels of specific analytes (organochlorine pesticides and PCBs). Sensitive detection methods, e.g. electron capture, require minimal interferences from not only solvents and reagents, but also from the column itself. Cleanup columns must be pretreated, usually with thermal and solvent desorption procedures. Elevated temperature bake-out removes adsorbed water and volatile organics from the active surface of the column packing material. Prior to sample application the column is washed with solvent (usually the same solvent to be used as eluant) to remove nonvolatile organics, and to establish equilibrium between mobile and stationary phase.

Other interferences are method specific, and involve factors such as co-elution of two or more analytes. Thus DDE is not separated from most PCB isomer mixtures, and elutes as an interference peak in a PCB fraction.

Recovery Data--

Recovery data have been reported by several investigators for organochlorine pesticides, PCBs and PBBs. In general, recovery ranges from 85-100% (total recovery) for these analytes from adipose (55,55) and blood samples (36,42,110). High recoveries (>85%) were also reported for phthalate esters from adipose tissue (128). Most of this data was obtained from clean-up procedures using Florisil or silicic acid/Celite columns.

Precision--

Using Florisil columns for cleanup of OCP/PCB samples, reported reproducibility was in the range of 1-2% for spiked adipose extracts (133). Plasma analysis of OCPs gave standard deviations of roughly 4-15% using Florisil cleanup (42). The analysis of DDE and PBBs from serum samples (36) gave %RSDs for recovery studies of 3-5%. A precision for analysis of PCBs using Aroclor 1260 as reference standard has been quoted (129). In general, better precision has been obtained for single component analytes (e.g., individual, OCPs), than for multicomponent analytes (e.g., PCBs).

Simplicity, Cost and Throughput--

Most columns used for sample clean-up employ readily available and inexpensive packings such as Florisil, silicic acid or alumina. Columns are usually dry-packed in glass columns, without the need for sophisticated packing techniques. All utilize gravity-flow, and, owing to their inherent low resolution, require only minimum operational expertise. The lack of instrumentation, and the ease of fraction collection allow for the operation of several columns at once. Thus many samples can be processed simultaneously by a single operator.

Recommended Method/Analyte Correlation Parameters--

Most nonpolar and semipolar compounds can be fractionated using column chromatography. Compounds which adsorb strongly to the column material cannot be recovered well.

Thin Layer Chromatography

Principle of the Technique--

Thin-layer chromatography (TLC), when used as a cleanup procedure, functions as cleanup column chromatography. The sorbent, usually is streaked with the sample containing the desired analytes. Development of the chromatogram is conducted in such a way that the analyte(s) are carried away from the origin to a distance equivalent to R_f values ranging from 0.2-0.8. In this fashion, separation from materials possessing different coefficients of adsorption (i.e., those materials of significantly greater or lesser polarity) is effected. In the case of silica gel TLC, more polar matrix components are retained at or near the origin, nonpolar components migrate with or near the solvent front, and the compounds of interest elute in the middle region of the chromatogram, along with compounds of similar polarity. The TLC region of interest is then removed from the plate, and the components removed by extraction with strong solvent.

Biological Matrices--

TLC has been used as a cleanup procedure for a variety of biological and environmental matrices, including adipose tissue (75,80,128,129), plasma (110), milk (131), urine (131) and hair (101). Silica gel and alumina plates were used most commonly, although Kieselguhr was reported in one instance (75).

Chemicals Analyzed--

In the majority of cases studied, target compounds consisted of organochlorine pesticides, and/or PCBs. The former includes such common pesticides as Dieldrin, DDT, Endrin, hexachlorobenzene, Chlordane, heptachlor, etc. The PCB group possess thin-layer chromatographic properties similar to the pesticides, and required subsequent separation; usually by GC, prior to analysis.

In one instance (128), TLC was used in a cleanup step for confirmatory analysis of phthalate esters from human adipose tissue.

Method Interferences--

As commonly practiced, TLC represents a low resolution chromatographic procedure, and thus is better suited for the separation of matrix components of widely differing polarities. Interferences then consist of molecules of similar properties such as PCBs and organochlorine pesticides. In at least one report (75), the difficulty of achieving reproducible retention values due to the "uneven quality of the adsorbent" was noted and represents a potential method drawback.

Concentration Factor - LOD--

As the TLC applications were limited, to cleanup procedures, limits of detection are not applicable.

Recovery Data--

From the literature reviewed, no recovery data were cited for TLC procedures used for cleanup of crude sample. In virtually all cases, the cleanup step was utilized in obtaining sample for confirmatory analysis by, usually, GC. The confirmation was qualitative in nature, and hence no attempt to rigorously define recovery was made. In each case, TLC scrapings were extracted with a solvent system stronger than the TLC developing solvent, to maximize sample recovery.

Precision and Accuracy--

No data are available for quantitative measurement parameters since cleanup was applied only for qualitative sample confirmation.

Simplicity, Cost and Sample Throughput--

TLC as a cleanup procedure is inexpensive, both in terms of equipment and supplies, and labor. The ability to conduct multiple separations on a single TLC plate and to develop multiple plates at once, without significant analyst time, make for high throughput potential. However, these advantages are offset by the procedure's limitations. Quantitative analysis by TLC is not as useful as other techniques such as HPLC, and in many cases, sufficient amounts of material cannot be separated to allow subsequent analysis.

Recommended Method/Analyte Correlation Parameters--

Information is not available on sufficient numbers of compounds to suggest a correlation, although polarity and volatility are probable candidates.

Partition coefficients between two immiscible solvents ("p-values") have been studied with regard to pesticide identification. For a further discussion see Reference 21. Retention indices or specific retention volumes in gas chromatography would provide similar information.

DERIVATIZATION METHODS

Derivatization is the modification of chemical compounds and is often used to eliminate problems in qualitative and quantitative GC analysis. For organic compounds, derivatization involves the replacement of an active hydrogen or a polar functional group with a less reactive constituent. This effectively reduces compound polarity and decreases the possibilities of hydrogen bonding to give increased volatility as well as chemical stability. For gas chromatography, derivatization minimizes solute-sorbent interaction resulting in reduced irreversible adsorbtive losses, reduced peak tailing and improved compound separation.

Since chemical derivatization is an additional step in a procedure, it is a potential source of error which could seriously affect the quantitative precision and accuracy of an analytical scheme. For this reason, a thorough knowledge of the reactions used and the factors which influence results is necessary. Ideally, all derivatization reactions should be efficient, quantitative, reproducible, rapid, and easy to perform; and should produce a stable, volatile derivative which is free from contaminants and other experimental artifacts. Unfortunately, no reaction satisfies all these requirements, and existing methodologies are compromises which satisfy the most important criteria for a given experiment.

Although numerous derivatization procedures are available, only the most common will be discussed. These include esterification, silylation, and acylation. Additional information is available in abundance in the chromatography literature as well as in texts (134-136).

Esterification or Ether Formation

Principle of the Technique--

Esterification of organic acids increases volatility and protects polar acid groups during subsequent reaction. Methyl esters are the most widely used product. Higher alkyl esters have also been employed both to provide a less volatile derivative and to distinguish derivatives from naturally occurring methyl esters. For simplicity, only methylating reactions will be discussed, however, the preparation of other alkyl esters is essentially equivalent using higher homologues of the derivatizing agent.

Methylation with diazomethane is the most common esterification technique. Diazomethane, which is a yellow colored gas reagent, is formed from the reaction of N-methyl nitrosoguanidine with a base such as sodium hydroxide. For derivatization, diazomethane is gradually added to an ethereal solution of the acid compound until esterification is complete. Generally, this is done until a yellow color persists signaling that excess reagent is present. Since the reaction between diazomethane and organic acids is rapid (usually less than one minute), color is an acceptable method for determining the endpoint of the reaction. Reagent is either added as an organic solution or is bubbled through the sample extract as a vapor. This latter method is somewhat safer and also introduces fewer reagent contaminants. Anhydrous conditions are recommended to prevent diazomethane from reacting with water. Diazomethane derivatizes the hydrogen form of organic acids, therefore the reaction solution should be acidified if strong acids such as trichloroacetic acid are to be derivatized.

Diazomethane esterifications are simple, rapid and for the most part quantative. Although the higher homologues of the reagent (i.e., diazoethane, diazopentane) behave in a similiar manner, the reaction is less facile. The major disadvantages to this reagent are that both diazomethane and its precursor, N-methylnitrosoguanidine, are toxic and carcinogenic. Diazomethane is also explosive and highly reactive. Since polymer formation may occur during prolonged storage, it is best to perform derivatizations immediately after preparing the diazomethane. Problems associated with contamination in reagent blanks have been reported, however, these problems are generally eliminated if the reagent is distilled prior to use.

Biological Matrices--

Reaction with diazomethane or its higher homologues has been used to derivatize extracts from blood (69,70,83,84,92,113,114), urine (69,72,82,83,84, 111,113,137), and adipose samples (84).

Chemicals Determined--

Diazomethane or its higher homologues can be used to esterify any organic acid which is present in a sample extract as the free acid. Sulfonic, phosphonic, and carboxylic acids as well as organic phosphates and acid phenols are amenable to derivatization. The less acidic phenols do not give quantitative or reproducible yields. Esterification of dicarboxylic acids is not always complete with mixtures of the mono- and diesters being formed. Citations have reported the derivatization of alkylphosphates (82,84), 2,4-dichlorophenoxy acetic acid (76,78), *p*-nitrophenol (85), mandelic acid (111), phenylglycolic acid (111), chlorinated phenols (56,115), pentachlorophenol (87,111,137) and hexachlorophene (108). Secondary reaction products were reported during analyses of mandelic and phenylgloxylic acid (111).

Recovery Data--

Standards and controls are usually derivatized using the same reaction conditions as for samples, therefore absolute recovery data for the derivatization step is not generally available.

Precision--

No data was available in the reviewed articles for the derivatization reaction.

Simplicity, Cost, and Sample Throughput--

Derivatization with diazomethane is a rapid simple procedure once the reagent has been prepared and as many as 30 samples/hour can be processed. However, since both the starting material and the reagent itself are toxic and carcinogenic, extreme care must be taken both preparing diazomethane and derivatizing samples. Special equipment is required for diazomethane preparation and a toxic lab or a very good fume hood must be used for all sample handling. These restrictions significantly limit sample throughput and increase the cost of sample processing.

Recommended Method/Analyte Correlation Parameter--

Reaction with diazomethane can be performed on any organic acid which has a pKa less than 8. Weaker acids and dicarboxylic acids do not derivatize well.

Extractive Alkylation

Due to the dangers associated with esterification using diazomethane, as described above, alternative derivatization reactions have been developed. Recently, a technique known as extractive alkylation or ion-pair extraction, has been employed in the analysis of alkyl phosphates in urine (138). The alkyl phosphate is extracted as an ion-pair with a lipophilic quaternary ammonium cation into a nonpolar solvent such as methylene chloride which contains the alkylating agent, pentafluorobenzyl bromide. The poor solvating ability of the nonpolar phase renders the alkyl substrate highly reactive toward nucleophic displacement. The derivative thus formed is stable to gas gas chromatography and may be detected and quantified with an ECD.

Human urine (138) has been fortified with alkyl phosphates at levels of 0.1 ppm. At this level, coextracted compounds naturally present in urine have presented minimal interferences and cleanup of the extract has not been necessary.

The method is simple and relatively rapid. The extraction/derivatization requires approximately 30 minutes, with some additional time for sample concentration, if necessary. The chromatography is most likely the step which limits the number of analyses per day.

Silylation

Silylation is the introduction of a silicon-containing group into an organic molecule usually in substitution for an active hydrogen. Trimethylsilyl (TMS) derivatives are the most common modifiers having the chemical structure, $Si(CH_3)_3$. Preparation of possible silyl derivatives is represented by the scheme:

$$\begin{array}{lcl} \text{-OH} & & \text{-O-Si(CH}_3)_3 \\ \text{-COOH} & & \text{-COO-Si(CH}_3)_3 \\ \text{-SH} & \xrightarrow[\text{donor}]{\text{TMS}} & \text{-S-Si(CH}_3)_3 \\ \text{-NH}_2 & & \text{-NHSi(CH}_3)_3 \\ \text{-NH} & & \text{-N-Si(CH}_3)_3 \end{array}$$

and if the enolized carbonyl group is included, then all the common polar groups are represented. The general order for decreasing ease of reaction is: alcohols (primary, secondary, then tertiary), phenols, carboxylic acids, amines (primary, secondary, then tertiary), and amides.

Conditions necessary for TMS derivatization have been studied extensively and, for the most part, are well understood. For high derivative yield, a reaction scheme must provide: (a) a derivatizing agent which is more basic than the compound to be derivatized; (b) a polar reagent solvent capable of stabilizing the charged reaction intermediate; and (c) a mechanism for removing spent reagents and hydrogen ions as the reaction proceeds. A variety of reagents, catalysts, and reaction conditions have been employed to meet the above specifications.

Since most TMS reagents are more volatile than their associated derivatives, they should not interfere with chromatographic analysis, and in most cases, no attempt is made to separate the reaction products from the reaction mixture. However, during mass spectrometry, reagents often decompose in the ion source to form SiO_2 causing problems such as tailing, baseline drift, and variable sensitivity. In these cases, it may be preferable to isolate derivatives from the reaction mixture via distillation. For the more stable derivatives, low molecular weight alcohols are sometimes added to the reaction mixture to quench unreacted agents and prevent by-product formation.

The main advantages of TMS derivatives are their facile preparation and their ability to modify a variety of functional groups. In addition, they give characteristic mass spectral fragmentation patterns useful for compound identification.

Silylation is often considered an unfailing means of improving GC separations; however, no technique is without limitations. Trimethylsilyl derivatives are unstable in water, requiring that anhydrous conditions be maintained throughout the entire analytical procedure. Carboxylic acid derivatives are the least stable with even the slightest trace of water inducing hydrolysis. Derivatives formed from secondary and tertiary functional groups tend to be more stable, suggesting that steric hindrance may prevent hydrolysis.

t-Butyl silyl reagents have been developed which form sterically hindered products that are relatively stable towards water. These reagents allow silylation to occur even when water is present in the reaction mixture.

Silylated compounds can cause some problems during chromatography. When using a flame ionization detector, silicon dioxide may be deposited on the electrodes which can alter detector response and lead to reduced sensitivity. Ghost peaks have been reported during gas chromatography of silylated phenols. Finally, it should be mentioned that the reactivity of different functional groups toward silylation varies widely. Because of this, reaction time and conditions must be carefully controlled in order to achieve quantitative, reproducible yields without producing side reactions or degradation products.

HMDS has been used to derivatize p-nitrophenol in urine samples with an on-column reaction technique (99). Precision of the entire analytical procedure reported as % relative standard deviation was less than 5%. Data was based on triplicate processing and analysis of four urine samples.

Urinary acids have been analyzed by gas chromatography after conversion to the trimethylsilyl derivatives using BSTFA:TMCS (N,O-bis(trimethylsilyl)-trifluoroacetamide:trimethylchlorosilane) or BSA:TMCS (N,O-bistrimethylsilyl-acetamide) (85).

Simplicity, Cost and Sample Throughput--

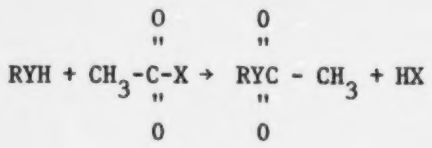
Using an on-column reaction technique as reported (99) minimizes the effort and cost of the derivatization procedure. The only manipulation required is addition of the reagent to the sample extract.

Recommended Method/Analyte Correlation Parameter--

Information from chromatography supply houses can be useful in deciding which reagent to use for a given compound or compound class. As discussed above, a wide variety of functional groups can be derivatized using these reactions.

Acylation

Acylation is a common derivatization technique for hydroxy, thiol, and amino compounds which, unlike silylation, can modify even the unreactive tertiary amino group. The chemistry of acylation usually involves the introduction of an acetyl group into the molecule by substitution for a replaceable hydrogen. The reaction proceeds according to the scheme:



$$RYH + CH_3-\underset{\underset{O}{\shortparallel}}{\overset{\overset{O}{\shortparallel}}{C}}-X \rightarrow RY\underset{\underset{O}{\shortparallel}}{\overset{\overset{O}{\shortparallel}}{C}} - CH_3 + HX$$

The reagents most commonly used include: acid anhydrides, acid chlorides, and some substituted derivatives, such as acylated imidazoles, amides, and phenols.

Acylhalides are highly reactive and capable of derivatizing any of the specified functional groups including amides and sterically hindered moieties. Unfortunately acid halides are produced during derivatization necessitating the presence of a base in the reaction mixture in order to drive the reaction to completion. Excess acid and halide salts can cause chromatographic problems, however removal is difficult, thus limiting the applicability of these approaches.

Acid anhydrides are usually easier to remove at the end of a reaction. Derivatization yields a clean product since only the acylated product and organic acid are formed. However, anhydrides are less volatile than the corresponding halides and may interfere with the chromatography of more volatile products. In addition, the acidic medium makes this reagent unsuitable for acid-sensitive compounds.

Acylation reagents that form basic leaving groups, i.e., acylation imidazoles and acetamides, are more satisfactory for derivatizing acid sensitive molecules. The basic leaving group associates with the displaced hydrogen to form a neutral by-product. For this group of derivatives, as well as the other reagents, the type and amount of solvent frequently has a significant influence on reaction yield.

Because of their high reactivity, excess reagents may have an adverse effect on subsequent analysis, and, therefore, should be removed. Common problems include: irreversible alterations of the chromatographic column, corrosion or other damage within the chromatographic or GC/MS system, and re-derivatization of non-volatile residues at the top of the GC column. Since the acid anhydrides are the easiest to remove, and do not require basic acceptor molecules (like the acid halides), they have become the most common derivatizing reagent.

Halogenated acyl derivatives are commonly used in trace organic analysis. Most polyhalogenated reagents are either acid anhydrides or chlorides, whose reaction advantages and disadvantages parallel those described above. Fluorinated derivatives are the most widely used due to their increased volatility and high electron capture detector (ECD) sensitivity. Some of the most common reagents of this type contain the trifluoroacetyl (TFA), heptafluorobutyl (HFB), pentafluoropropionyl (PFP) and pentafluorobenzyl (PFB, see earlier discussion on extractive alkylation) moieties. Two TFA-amide reagents have been developed which form basic amide leaving groups. These reagents produce reaction by-products which are very volatile and easily removed. They also react under mild, non-acidic conditions. Unfortunately excess reagent is not easily removed and thus chromatographic problems may prevail.

Acylated derivatives for both hydroxy and amino groups are easily prepared and give higher yield than the various methylating procedures. For this reason they are the method of choice for both functional groups.

Biological Matrices--

Acylation reactions have been used to derivatize organic acids extracts from blood (38,116), urine (38,88), and adipose (38,47) samples, amines in urine (57,81,117,118) and plasma (97), as well as estrogens (phenols) in urine (33,73).

Chemicals Determined--

Acylation reactions have been applied to the analysis of dichlofenthion (38), hexachlorophene (16), pentachlorophenol (47), chlorophenols (33), 1-naphthol (88), 3,3'-dichlorobenzidine (139,140), and tocainide (97).

Method Interferences--

Solvent partitioning (47,88) and column chromatography (47) techniques have been used to remove interferences after derivatization.

Recovery Data--

Essentially 100% conversion was reported for the 1-naphthol to 1-naphthyl chloroacetate derivatization reaction using chloroacetic anhydride as the derivatizing reagent (88).

Precision--

Analysis of duplicate samples gave a RSD of 15-20% using procedures for the analysis of hexachlorophene in blood (116).

Simplicity, Cost, and Sample Throughput--

Acylation is a simple reaction requiring no specialized glassware or equipment. Even when using a solvent partitioning step to remove excess reagent approximately 20 samples can be processed with one manhour of labor.

Recommended Method/Analyte Correlation Parameters--

Acylation can be used as a derivatization method for any organic compound with a replaceable hydrogen including alcohols, thiols, and amines. Compounds with the most reactive nitrogen are most readily derivatized however relatively unreactive tertiary amino and alcohol groups can be derivatized successfully.

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SECTION 12

MASS SPECTROMETRY

INSTRUMENTATION

Principle of the Technique

Mass spectrometry, one of the most powerful analytical techniques available today, yields detailed structural information about organic compounds and accurate elemental analysis of solid state samples. It is often possible to positively identify an unknown compound based on a detailed interpretation of the mass spectrum alone.

The mass spectrometer produces charged particles from the sample molecules. These particles consist of a parent or molecular ion and ionic fragments of the original molecules. The ions are then sorted and detected according to their mass-to-charge ratio (m/e). The instrumentation required to generate the mass spectrum includes an inlet system for vaporization of the sample, an ionization source where the sample is ionized, a mass analyzer which separates the ions according to their mass-to-charge ratio, and finally, a detection system.

A number of types of ionization sources are available. The first and still most commonly used source is the electron ionization (EI) source in which sample molecules are bombarded by electrons boiled off an emitting filament at 70 eV. Ionization of molecules in this manner is a rather harsh method which results in much fragmentation of the molecules. These fragment ions provide valuable information about the structure of the molecule. However, many compounds are so efficiently fragmented by electron ionization that no appreciable population of molecular ions remains to give an indication of the molecular weight. To supply this information, other, less energetic ionization sources have been developed.

One of the methods in routine use is chemical ionization. Using this ionization technique, a reagent gas is introduced and ionized by interaction with an emitting filament. The compound of interest is ionized by gas phase chemical reaction with the ionized particles which exist in this ionized reagent gas plasma. Depending on the choice of reagent gas, various reactions may occur, among them charge exchange (in which the molecules of the compound of interest become charged by interaction with the reagent gas plasma with minimal transfer of energy), proton addition, proton abstraction, and chemical reaction which results in addition of groups larger than a proton. Since the reactions occur with far less transfer of energy than interaction with a filament at 70 eV, less fragmentation is encountered. Thus, chemical ionization techniques are often employed in an attempt to obtain molecular weight information.

An additional mode of ionization called field ionization requires the use of high electric fields, of the order of volts per angstrom; the molecules are ionized by quantum mechanical tunneling, and the energy imparted in the process is minimal so that molecular ions are frequently obtained.

A related mode, field desorption, allows spectra to be generated from very labile compounds of extremely low volatility. The source is similar to the field ionization source; the sample is applied to the emitter, a wire upon which needles are grown. When a current is applied, the sample molecules migrate to high field regions at the points of the needles and are ionized.

Several other techniques can be used to generate ions from the surface of a solid sample. Among these "soft-ionization" techniques are the use of a Cf-252 plasma desorption source and a laser desorption source.

A new ionization technique which is currently under investigation in many laboratories is fast atom bombardment in which molecules of the compound of interest are ionized by interaction with a plasma of excited atoms (argon or xenon are typically used). Using fast atom bombardment, great success has been obtained with the ionization of molecules which have been very difficult or impossible to vaporize and which, therefore, could not be ionized by other techniques.

Ions can also be produced by a vacuum spark. Using spark source mass spectrometry, it is possible to cover the majority of the elements in a matrix in a single analysis, detect these elements down to very low levels of concentration with approximately the same levels of sensitivity for all elements, and with linearity over a wide concentration range. The vacuum spark is used to produce the ion beam in this mode of analysis. Since the ion current output as a spark source is very erratic, an integrating form of detector is required. By using a suitable geometry of the analyzer, it is possible to bring all the ions to focus simulataneously in a plane, allowing the use of an ion-sensitive photoplate as a detector. Analyses covering limits of detection in the 0.01 to 0.001 ppm range may be conducted. Operation in these ranges makes spark source mass spectrometry very valuable for the trace analysis of semiconductor materials.

The two major types of mass analyzers which are commercially available today are magnetic sector quadrupole and instruments. To achieve increased resolution, the electromagnet may be preceded or followed by an electrostatic sector to perform an energy separation of the incident beam of charged particles, thus providing mass resolution to four or more decimal places, allowing accurate determination of elemental composition. A quadrupole separates ions according to their mass-to-charge ratios by utilizing an electric filtering arrangement consisting of four accurately made and aligned metal rods, energized with rf and dc potentials. The rods which are diagonally opposite one another are connected electrically. Between the two pairs of rods is a constant voltage upon which a varying radiofrequency voltage is superimposed. Variation in these parameters allows an ion of any selected mass-to-charge ratio to traverse a path from the ion source through the analyzer. All other ions do not traverse this stable path and are deflected into the rods, discharged, and not detected. A systematic sweep of this radiofrequency voltage allows an entire mass spectrum to be scanned.

Both the magnetic sector mass spectrometer and the quadrupole may be interfaced with a gas chromatograph. However, there is a basic incompatibility between the two techniques. The mass spectrometer typically operates at pressures of 1-6 torr or lower. The effluents from a gas chromatograph are at pressures well above atmospheric. There are two ways by which the two techniques may be coupled: the flow from the gas chromatograph may be lowered, or some mechanism can be employed to preferentially remove carrier gas from the gas chromatograph effluent and thereby lower the pressure and enrich the flow in analyte. Both approaches have been employed.

The above represents a short summary of the common types of mass spectrometer systems available. A specialized field of mass spectrometry has evolved using the ability of the mass spectrometer to separate isotopes of a given element and to determine the masses of those isotopes. Two types of mass spectrometers are typically used in this mode of analysis. A stable isotope enrichment mass spectrometer handles low molecular weight gaseous samples, is generally dedicated to measurement of one pair of isotopes, and yields the best precision for isotope ratios of any type of instrument, while a thermal ionization spectrometer handles samples which are not extremely volatile and produces abundance ratios for a wide range of the heavier elements. Stable isotope enrichment mass spectrometers are capable of detecting very small differences in the isotope abundance ratio of a number of elements and can thus measure natural variations which arise from differences in the biological origins of the substances which contain these elements. The implications for geochemical and environmental analyses are major. Thermal ionization mass spectrometers are designed to measure isotope abundance ratios of nonvolatile elements by combustion upon a filament. Using thermal ionization and multiple filaments, it is possible to adjust temperatures so that one element is selectively analyzed in the presence of a mixture. This type of analysis is typically employed in the determination of atomic weights and nuclear constants, as well as accurate age determination and pollution monitoring, especially in the analysis of inorganic materials.

Chemicals Determined

Chemicals analyzed by mass spectrometry range from low molecular weight gases to high molecular weight polymers, from volatile organic materials to inorganic salts. To illustrate the flexibility of the technique, some pertinent examples will be cited.

Spark source mass spectrometry was used to analyze trace metals in tissue samples of miners. A set of twenty elements was selected for analysis by this technique: aluminum, arsenic, beryllium, bismuth, cadmium, chromium, cobalt, copper, germanium, iodine, lead, magnesium, manganese, molybdenum, selenium, tellurium, thallium, titanium, vanadium, and zinc (1).

Isotope ratio mass spectrometry was used in the analysis of lead in blood (2); a study of maternal and infant cord blood was also performed. Simultaneous blood and facial hair levels of lead were also determined by isotope ratio mass spectrometry (3), and the technique was also used to analyze lead in feces, urine, blood, saliva, and gastric, pancreatic, and biliary secretions.

Field desorption mass spectrometry and stable isotope dilution was used to determine lithium, rubidium, magnesium, and strontium in urine (4) of a multiple sclerosis patient.

Many references are available in which gas chromatography/mass spectrometry (GC/MS) was used to identify organic compounds in biological matrices. These citings include the determination of *p*-dichlorobenzene in adipose tissue and blood (5), identification of polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans in tissue (6,7) and polychlorinated dioxins in plasma of occupationally exposed workers, polychlorinated terphenyls in human adipose tissue (8) identification of Mirex residues in human adipose tissue (16), analysis of 1,4-dioxane and its metabolite, β-hydroxyethoxyacetic acid, in blood (9) and urine (9,10), and identification of organophosphorous pesticides and their metabolites in tissues, urine and blood (11). Mild chemical ionization detection was used to assay N-nitrosodimethylamine by gas chromatography/high resolution mass spectrometry in human plasma at pg/mL levels (12). Antipyrine, 4-hydroxyantipyrine, 3-hydroxymethylantipyrine and norantipyrine were determined in urine by gas chromatography chemical ionization mass spectrometry (13).

The urine of men employed in shoe factories and various leather industries was studied by GC/MS for the presence of n-hexane, cyclohexane, and their isomers, since polyneuropathies described in these workers have been attributed to the inhalation of C5-C7 aliphatic hydrocarbons which are generally used as solvents for leather adhesives (14). Gas chromatographic/mass spectrometric confirmation was used to trace the metabolism of toluene in man by assaying o-, m-, and p-cresol in urine (15). Endogenous ethanol was determined in blood and breath of healthy abstaining subjects by GC/MS with deuterium labelled species as internal standards (24). GC/MS was used to identify N-methylsaccharin as an interferrant in the GC analysis of urine samples from workers exposed to 2,4,5-trichlorophenoxyacetic acid (25). The fate of orally administered valproic acid was followed in serum and urine by GC/MS (26). Several new metabolites were identified.

In workers occupationally exposed to styrene, excretion of 4-vinylphenol in urine was verified by gas chromatography/mass spectrometry (17). Blood was analyzed for inhaled styrene by gas chromatography/mass spectrometry (21), and pharmacokinetic modeling was performed. Circulating plasticizers in the blood of surgical patients were identified and quantified by the use of gas chromatography/mass spectrometry (22).

Most of the studies cited above were compound-specific studies, that is, a specific compound was sought and either its identification was confirmed or the compound or series of compounds was identified and quantified by mass spectrometry, usually coupled with a gas chromatograph. Mass spectrometry can also be used as a survey instrument.

GC/MS in the single ion monitoring mode has been used to identify chlorophenols in human urine (28) and to determine chlorophenols in human urine in combination with high performance liquid chromatography (29). The study of volatile components in the body fluids of subjects has potential as a means of studying disease. For example GC/MS has been used to confirm the identity of volatile low-molecular-weight aldehydes, ketones and alcohols in blood and cerebrospinal fluids of patients suffering from chronic encephalopathy (30). Metabolic screening using GC/MS has been applied to organic acids in human plasma (31), organic acids in amnionic fluids (32), identification of previously unknown polar and metabolites in human urine (33), to identification of volatile organic acids in urine from maple syrup urine disease (34), and to identification of urinary metabolites from patients with propionic acidemia or β-ketothiolase deficiency (35).

Gas chromatographic/mass spectrometric identification has also been used to measure the chemical inhalation exposure in an urban population in the presence of endogenous effluents (7), to quantitate organochlorine industrial chemicals and pesticides in human blood (36) and adipose tissue (37), to screen blood, urine and tissue samples for 30 volatile compounds (38). GC/MS has been used for quantitation of and confirmation of organic phosphate triesters in human adipose tissue (20). Trace levels of compounds from human adipose tissues and blood samples resulting from exposure to technical chlordane have been identified (39) and GC/MS trace analysis of herbicidal acids from urine has been developed (40). Toxic-substance screening for polychlorinated acidic substances has been accomplished using negative chemical ionization mass spectrometry (41).

A comprehensive sampling and analysis by GC/MS has been developed for volatile halogenated hydrocarbons in breath, blood, urine, air, and drinking water (27).

Of the many possible dioxin isomers, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has received much attention because of its extreme toxicity and possible toxic effects from environmental exposure. Methods for analysis must be able to determine ppt levels in very complex samples. Analytical extraction, a multi-step cleanup procedure and capillary column gas chromatography/high resolution mass spectrometric quantitation have been used to detect low concentrations (pg/g) of TCDD in human milk (19). A mean % recovery of 80% for 2.5 to 10 ng $^{37}Cl_4$-TCDD internal standard and a mean % accuracy of ±23% for 1 to 1250 pg TCDD were reported.

Methods of Quantitation

Various methods of quantitation can be employed, depending upon the mass spectrometric technique employed. Most are based upon the use of external or internal standards. When external standard quantitation is used, the instrument response is calibrated with some standard reference material, and the concentration of the compound of interest in the media studied is calculated by comparing the peak area measurement of standard to the peak measurement of the analyte obtained when the sample is analyzed. Often a calibration curve is obtained by using concentrations of standard which span the concentration range of interest, using a linear regression or least squares correlation, and calculating interpolated values on the basis of the equation obtained for the correlation. Rappe et al. (7) used the external standard method to identify and quantify polychlorinated dioxins and dibenzofurans in human plasma and tissue samples from exposed populations. The detection levels were in the ppt region.

When GC/MS analysis is used, quantitative data may be obtained in either the full scan mode or in the selected ion monitoring mode, and the basic methods are the same. The instrument can be calibrated absolutely: various concentrations of standard material can be analyzed in replicate, and the instrument calibrated by repeated observation of an absolute response (peak height or peak area) to the quantity of material injected. Alternatively, internal or external standard (or standards) can be used, and the instrument calibrated by observing a relative response between known quantities of standard and of compound of interest. A response factor can be calculated to allow for the difference in instrument response between the standard and the compound of interest. During analysis, this response factor is used to calculate the amount of the compound of interest observed. The response factor can be calculated at a single concentration or over a range of relative concentrations. The standard chosen can vary; often an isotopically labeled analog of the compound of interest is used, sometimes a homolog, and (when quantitative analysis of a series of compounds, related or unrelated, is desired) a compound totally unrelated to the compound(s) of interest may be employed.

Internal standards are added to a sample before it is processed so that it may be used to correct for losses incurred in subsequent extraction and purification steps and to provide a known quantity against which to measure the analyte. External standards are chosen which are similar to the analyte, but which would not occur as a contaminant in the sample. Stable isotopes are used frequently for this purpose. Stable isotope dilution is also a valuable technique used for quantitation in isotope ratio mass spectrometry: a quantity of pure isotopic spike is added to an aliquot of the sample of the biological medium, and the biological sample is put through the usual workup. Since the concentration of the element of interest is made from the isotopic ratio of the mixture once the spike has been added, high procedural recovery is not essential. The concentration can be verified by processing replicate samples, sometimes using different workup methods. A nonisotopic compound, 4,6-dibromo-o-cresol, has been used as an internal standard for the identification and confirmation of chlorophenol residues in human urine down to the 1.0 pmole/mL level (28). A stable isotope [$^{15}N_2$] of N-nitrosodimethylamine (NDMA), was used as an internal standard in the assay of NDMA in human plasma (12). The limit of quantitation was reported at 31 pg/mL with two internal standards (early versus late eluting) were used to quantitate volatile compounds in human biofluids (38).

Limit of Detection

The limit of detection varies with the mass spectrometric technique used. In general, when the full scan mode electron ionization is used, the instrumental limits of detection range from approximately 5 to 50 ng, depending on the compound and chromatographic conditions. In the selected ion monitoring mode, the limit of detection can often be decreased to the low nanogram range, sometimes even to low picogram range, again depending upon the compound and the chromatographic conditions. Estimated limits of detection for the range of mass spectrometric techniques are given in Table 12-1.

In cases where the mass spectrometer is used to confirm the identification of a compound, no limit of detection for the mass spectrometric technique is usually reported. In many cases, the mass spectrometer is used for the purpose of confirming a compound identification, but the actual quantitative analysis is performed by gas chromatography.

Precision

Although the % RSDs for electron ionization capillary GC/MS are generally about 25%, values of 5% or better have been reported (12).

Accuracy

The accuracy of a method for determination of an analyte in a complex biological matrix is generally defined by the use of spiked samples and is not specific for the instrument only. Harless et al. (19) report a mean % accuracy of ±23% for 1 to 1250 pg TCDD in human milk.

Simplicity, Cost, and Sample Throughput

The mass spectrometric technique is usually simple. Once the sample is prepared and introduced to the ion source of the mass spectrometer, the rest

TABLE 12-1. ESTIMATED LIMITS OF DETECTION (LOD) FOR SELECTED MASS SPECTROMETRIC TECHNIQUES

Technique	LOD
Packed Column GC/MS	
EI[a], full scan	25-50 ng, compound dependent
EI, SIM[b]	1-10 ng, compound dependent
CI[c], full scan	15-50 ng, compound dependent
CI, SIM	0.5-10 ng, compound dependent
Capillary GC/MS	
EI, full scan	5-25 ng, compound dependent
EI, SIM	0.1-5 ng, compound dependent
CI, full scan	5-30 ng, compound dependent
CI, SIM	0.1-5 ng, compound dependent
Spark Source	0.006-0.6 ng, element dependent

[a] Electron ionization

[b] Single ion monitoring

[c] Chemical ionization

of the analysis can often be automated or put under control of a data system. When the use of mass spectrometry is used solely to confirm the identification of a compound of interest, mass spectrometry is generally a very simple technique. Quantitative analysis by mass spectrometry is a more complicated process, in that calibration of the instrument by some method is required. The mass spectrometric technique is one of the most expensive analytical methods which the analyst might choose. The cost of the instrument can range from approximately $50,000 to more than $500,000. A mass spectrometer used frequently for qualitative and quantitative assays requires an experienced operator. In general, the mass spectrometer is not the analytical technique of choice when a cheaper method is adequate, and testimony to this fact is the frequency with which the mass spectrometer is used for identification of compounds of interest, while quantitative assays upon large numbers of samples are performed by gas chromatography (4,6,9,10,12,14,16). However, when the specificity of the mass spectrometric technique is required, there is no alternative.

Sample throughput is also widely variable, ranging from a few minutes to two hours or more for a complex assay requiring slow analysis by capillary gas chromatography. A further factor in sample throughput is instrument calibration, although if the instrument is very stable, calibration once a day may be sufficient.

Simplicity, cost, and sample throughput are thus difficult to evaluate because of the many variables. A general rule which can be applied is that most other analytical methods are less costly in terms of instrument purchase, and the use of mass spectrometry should therefore be considered only when the specificity of the technique is required to perform a given assay.

SAMPLE PREPARATION METHODS

Sample preparation for organic compounds are similar to those used for other analytical techniques such as GC and are discussed in detail in Section 11. In some cases, biological samples can be analyzed directly. The field desorption mass spectrometric analysis of Li^+, Ne^+, Mg^{2+} and Sr^{2+} was carried out on urine with little or no sample preparation (4). When a pharmacokinetic study of 1,4-dioxane in humans was performed, the samples of blood plasma and urine obtained were analyzed directly (7); the beta-hydroxyethoxyacetic acid metabolite was methylated before analysis. The advantages of direct analysis of the biological medium are obvious in terms of simplicity, cost, and sample throughput.

Acid Digestion

Principle of Technique--

When isotope ratio mass spectrometry was used to assay lead in blood, analyses were carried out in a low lead laboratory, essential to extremely accurate determination of relative abundances of lead isotopes (2). The sample preparation method essentially required complete digestion of the matrix in order to free the lead compounds: the blood sample was evaporated to dryness, 70% HNO_3 was added, and the sample was boiled until NO_2 no longer evolved. A lead isotope spike was added and lead was coprecipitated with $Ba(NO_3)_2$. The precipitate was washed and dried, dissolved in water, and the lead separated from the barium by deposition onto a platinum anode. The lead was dissolved with HCl, the solution evaporated to dryness, and the resultant solid was loaded onto a filament of a mass spectrometer.

Biological Matrices--

Since the matrix is essentially destroyed by this method, the method should be applicable to any biological matrix. A basically similar method for preparing blood and facial hair for the analysis of lead required that the hair be washed with soap and water and air dried before the addition of the lead spike and dissolution in concentrated nitric acid; the lead was subsequently extracted with dithiazone and treated as above (3).

Chemicals Determined--

Metal ions, which are impervious to the severe matrix-destruction methods employed, are the chemicals analyzed by this technique. Since the matrix is destroyed, no interferences other than other metals can remain.

Method Performance--

No concentration factor is reported (2,3). No limit of detection was reported. Recovery was estimated to be approximately 40% (2). Precision and accuracy for the analysis of lead in blood using isotope ratio mass spectrometry and the relative standard deviation of each ratio obtained was reported to be about 0.5% under favorable conditions of signal stability (2).

Simplicity, Cost, and Sample Throughput--

Sample throughput should be high; given the proper environment and equipment, many samples can be processed simultaneously.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

If a material is to be analyzed by mass spectrometric methods, several requirements must be met. If gas chromatography/mass spectrometry is to be used, the organic compound must be sufficiently volatile to pass through the gas chromatograph. Some components can be derivatized to increase volatility. Where a compound is not sufficiently volatile to be analyzed by one of the coupled chromatographic/mass spectrometric techniques and cannot be suitably derivatized, alternative methods of ionization (such as field desorption or fast atom bombardment) can be tried and may be successful. The mass spectrometric technique is also limited by the mass range of the available instrumentation. If metals analysis is sought, isotope ratio mass spectrometry can be used only if the distribution of the isotopes of the metal of interest is such that the metal can produce suitable isotope ratios--a metal with highly radioactive isotopes or a mono-isotopic metal could not be analyzed in this way. Spark source mass spectrometry can be used to analyze a much broader range of metals, as well as some anions.

A basic parameter which must be considered is cost. As discussed previously, mass spectrometric methods are quite costly in comparison to other techniques available. If the specificity of the mass spectrometric technique is required, there may be no alternative.

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SECTION 13

X-RAY SPECTROSCOPY

INSTRUMENTATION

Principle of the Technique

When an atom is excited by removal of an electron from an inner shell, it usually returns to its normal state by transferring an electron from some outer shell to the inner shell with consequent emission of energy as X-rays, that is, photons of high energy and short wavelengths in the order of tenths of angstroms to several angstroms. Eventually a free electron will be captured by the ion.

X-rays can be used in chemical analysis in several ways. One method uses the fact that the X-rays emitted by an excited element have a wavelength characteristic of that element and an intensity proportional to the number of excited atoms. Thus, emission methods can be used for both qualitative and quantitative work. The excitation can be carried out in several ways: by direct bombardment of the material with electrons (direct emission analysis and electron probe microanalysis) or by irradiation of the material with X-rays of shorter wavelength (fluorescent analysis).

A second method of X-ray analysis utilizes the differing absorption of X-rays by different materials (absorption analysis). Major discontinuities in the absorption of X-rays by an element occur when the energy of the X-rays become sufficient to knock an electron out of the inner levels of an atom.

A third method of using X-rays in analytical work is the diffraction of X-rays from the planes of a crystal (diffraction analysis). This method depends upon the wave character of X-rays and the regular spacing of planes in a crystal. Although diffraction methods can be used for quantitative analysis, they are most widely used for qualitative identification of crystalline phases.

In 1913, Moseley first showed the extremely simple relation between atomic number, Z, and the reciprocal of the wavelength, $1/\lambda$, for each spectral line belonging to a particular series of emission lines for each element in the periodic table. This relationship is expressed as

$$\frac{c}{\lambda} = a(Z - \sigma)^2$$

where a is a proportionality constant and σ is a constant whose value depends on the particular series.

X-ray emission and absorption spectra are quite simple because they consist of very few lines compared to the emission or absorption spectra observed in the visible or ultraviolet regions. This relative simplicity arises because the X-ray spectra result from transitions between energy levels of the innermost electrons in the atom. There are only a few electrons in these inner shells and the resulting energy levels are limited, thus giving rise to only a few permitted transitions. There is only one K shell. The L electrons are grouped according to their binding energy into three sublevels: L_I, L_{II}, and L_{III}; the complete M shell consists of five sublevels. X-ray emission or absorption spectra are dependent only on atomic number and not on the physical state of the sample nor on its chemical composition, except for the lighest atoms, because the innermost electrons are not involved in chemical binding and are not significantly affected by the behavior of the valence electrons. An X-ray fluorescence spectrometer needs an X-ray source, a means of dispersing the X-rays, and a detector. The conventional X-ray spectrometer consists of a high-intensity X-ray tube with a stabilized, high-voltage power supply; a multi-sample chamber that can be evacuated; a goniometer (for measuring the diffraction angle) with either a parallel-plate collimator or a slit system, depending on the X-ray optics; and a proportional or scintillation-counter detector, together with its associated electronics which include a DC power supply, scaler-ratemeter, linear amplifier, single-channel analyzer, and recorder. Many of the newer units have the output of the single-channel analyzer coupled to magnetic or paper-tape storage and a dedicated or time-sharing computer. Some of the most promising applications of X-ray spectroscopy are in the medical field (1,2). The most wide spread use of X-ray spectroscopy has been in metallurgy, but it is applicable to analyze metals, minerals, glasses, ceramics, polymers, liquids and biological matrices.

Samples can be handled as liquids in cells usually 5 mm thick, solvent is usually H_2O. Solid samples can be powders and they are usually ground to a mesh size finer than 200 mesh.

Chemicals Determined

X-ray spectroscopy is suitable for multielement analysis and a wide range of elements have been determined with these techniques. Among the elements reported are K (3), Ca (3), Zn (3-6), Fe (3), Sr (3-6), Cu (3,4,6), Pb (3,4), Se (3,6), Rb (3,6), As (4), Br (4,6), Mo (4), Ag (4), Sc (6), Cr (6), Co (6), Ge (6), Y (6), Nb (6), V (6), Th (6), Hg (6), Pl (6), Ir (6), Os (6), Cd (7) and Sn (8).

Method of Quantitation

Calibration plots (6) are prepared using standard materials which have been previously analyzed using other techniques such as AAS. In addition, internal standards have also been used (3).

Limit of Detection

The limit of detection (LOD) of several elements have been reported in the literature surveyed. Table 13-1 shows some of this data.

Precision and Accuracy

The precision of this technique has been reported to be approximately 10% RSD (3).

Simplicity, Cost and Sample Throughput

Although many elements can be analyzed simultaneously, this technique appears to be expensive. Research-type, single-channel X-ray spectrometers cost on the order of \$25,000 to \$50,000; control instrumentation can range in costs from a few thousand dollars for a portable analyzer to \$200,000 for a 15 to 20-element simultaneous analyzer with computer and automatic sample transports.

TABLE 13-1. LIMIT OF DETECTION FOR X-RAY SPECTROSCOPY

Element	Matrix	Limit of Detection (ppm)	Reference
Sc	Blood, urine	5×10^{-2}	6
Cr	Blood, urine, tissue	3.4×10^{-1}	6
Co	Blood, urine, tissue	7×10^{-3}	6
Cu	Blood, urine, tissue	1.8×10^{-1}, 0.5-0.2	6,3
Zn	Blood, urine, tissue	12.6	6
Ge	Blood, urine	2.8×10^{-2}	6
Se	Blood, urine	1.5×10^{-2}	6
Br	Blood, urine	1.4×10^{-2}	6
Rb	Blood, urine	1.0	6
Sr	Blood, urine	1.3×10^{2}	6
Y	Blood, urine	8.1	6
Nb	Blood, urine	1×10^{-1}	6
Th	Blood, urine	3×10^{-1}	6
Hg	Blood, urine	4.2	6
Pt	Blood, urine	2.0	6
Ir	Blood, urine	6×10^{-3}	6
Os	Blood, urine, tissue	7×10^{-1}	6
K	Blood	4	3
Ca	Blood	4	3
Fe	Blood	0.5-0.2	3
Pb	Blood	0.5-0.2	3
Zn	Blood	0.5-0.2	3

SAMPLE PREPARATION METHODS

Dry Ashing

See Section 4.

Freeze Drying

See Section 4.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

Biological samples containing metals can be analyzed simultaneously by X-ray spectroscopy. This technique has wide applicability and has been used to determine most elements of interest.

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SECTION 14

NUCLEAR MAGNETIC RESONANCE

INSTRUMENTATION

Principle of the Technique

Like other forms of spectroscopy (for instance, infrared and ultraviolet), nuclear magnetic resonance spectroscopy (NMR) deals with the measurement of energy gaps between states of different energy. However, unlike most other forms of spectroscopy, the phenomenon requires the presence of an external magnetic field and concerns nuclei rather than electrons. This is the origin of the terms "nuclear" and "magnetic" in nuclear magnetic resonance spectroscopy.

A strong magnetic field causes the energies of certain nuclei to be split into two or more quantized levels, owing to the magnetic properties of these particles. Transitions among the resulting magnetically induced energy levels can be brought about by the absorption of electromagnetic radiation of suitable frequency, just as electronic transitions are caused by the absorption of ultraviolet or visible radiation. The energy differences between magnetic quantum levels for atomic nuclei correspond to radiation energies in the frequency range of 0.1 to 100 MHz (wavelengths between 3000 and 3 m), which is in the radio-frequency portion of the electromagnetic spectrum.

The study of absorption of radio-frequency radiation by nuclei is called nuclear magnetic resonance; it has proved to be one of the most powerful tools available for determining the structure of both organic and inorganic species.

In common with optical spectroscopy, both classical and quantum mechanics are useful to provide an explanation of the nuclear magnetic resonance phenomenon. The two treatments yield identical relationships. Quantum mechanics, however, is more useful for relating absorption frequencies to energy states of molecules, while classical mechanics is more helpful in providing a physical picture of the absorption process and how it is measured.

Chemicals Determined

Nuclear magnetic resonance spectroscopy is applicable to a wide range of organic compounds; proton NMR is used to analyze any compound containing hydrogen. In addition, ^{13}C NMR is used for carbon containing molecules, ^{19}F NMR is used for fluorine containing compound and ^{31}P NMR for phosphorus containing molecules. Citations found reported analyzing intracellular free Mg^{+2} using ^{31}P NMR spectra of intracellular ATP in red blood cells (1), free amino acids, creatine, glucose, lactate, d-3 hydroxybutyrate, choline, citrate, fatty acids, CaEDTA and MgEDTA in whole blood using proton NMR (2), and glucose, alcohols, acetone, and organic acids in serum by proton NMR (3).

Method of Quantitation

Quantitation has been reported to be achieved by internal standard (3), calibration curve (2), and by calibration with other spectroscopic techniques (1).

Limit of Detection

Typical concentrations analyzed include glucose, alcohols and esters (3) in the mmol/L range, cholesterol (2) in the mM range, free Mg^{+2} (1) CaEDTA and MgEDTA in the range of ppm (2).

Accuracy and Precision

Analysis of ATP was reported to have precision of 8% and 2% (1). The accuracy of the NMR method was assessed by the determination of Ca^{2+} and Mg^{2+} concentrations in five samples of standard lyophilized plasma, Welcomtrol 1. The results were: Ca^{2+}, 2.50 ± 0.13 mM (2.55 ± 0.08 mM) and Mg^{2+}, 0.77 ± 0.04 mM (0.74 ± 0.02 mM) where the values in parentheses are based on peak areas obtained manually by peak weight and the others by computer integration. The coefficient of variation of the measurements was 5%, most of this variability being due to inaccuracies in the peak area measurements. The manufacturers independent determinations were reported to be: Ca^{2+}, 2.48 mM (cresolphthalein complexone), 2.44 mM (CaEGTA complexiometric titration), 2.46 mM (atomic absorption spectrophotometry) and for Mg^{3+} 0.78 mM (atomic absorption spectrophotometry). It is estimated that concentrations down to 10 μm can be detected with present ^{1}H NMR equipment, the four magnetically equivalent ethylenic protons effectively amplifying the metal concentration by a factor of 4 (2).

Simplicity, Cost and Sample Throughput

The operation of NMR instruments is rather complex. However, no significant sample preparation is required, very high purity is not normally necessary, but certain types of impurities such as paramagnetic substances must be excluded. The presence of solid impurities of any sort in the solution will degrade the homogeneity of the magnetic field and hence cause line broadening.

Cost of NMR instrumentation is very high, several hundred thousand dollars, depending on the features.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

Nuclear magnetic resonance (NMR) is used primarily to determine specific structural features in organic compounds. Samples should be free of magnetic interferences; the usual sample volume is about 0.2 to 0.5 mL, though capillary cells of 0.003 mL have been used. The usual sample concentration required is 5 to 20% w/v. For high resolution studies low-viscosity liquid samples are necessary, either as a pure liquid or as a diluted solution in an inert solvent.

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SECTION 15

THIN LAYER CHROMATOGRAPHY

INSTRUMENTATION

Principle of the Technique

Thin Layer Chromatography (TLC) represents a separation technique based on adsorption or partition processes, and employing a thin, flat bed of sorbent. Mobile phase is passed over the sorbent bed by gravity flow, centrifugal force, or, most commonly, capillary action.

The separation process is, in almost all cases, a partition phenomenon, since most of the commonly used adsorbents, such as silica, contain a layer of tightly adsorbed water and/or mobile phase. Whether liquid-liquid partition or liquid-solid adsorption, actual component separation depends on localized, instantaneous equilibria at each sorbent site. This equilibrium, between solute in the mobile phase and solute adsorbed or partitioned onto the stationary phase, is the essence of the general chromatographic process, and is never completely attained. The diffusion of the solute in the mobile phase increases the deviation from ideal behavior.

At constant temperature, the adsorption (or partition) process, in the idealized state, can be conceptualized via the use of adsorption isotherms (1). Over a wide concentration range of solute, the isotherms are parabolic, but approach linearity at low concentrations where the adsorption coefficient ($C_{adsorbed}/C_{desorbed}$) is constant.

In practice, the isotherm is curved, primarily because the adsorptive power of the surface generally falls off markedly even when only a small proportion of the adsorption sites is occupied, since the most active sites are occupied first. The lower the concentration of solute, the nearer the isotherm approximates the ideal form, and the less tailing is observed.

Sorbents most commonly used for TLC include silica gel, alumina, and cellulose. Silica is available in many forms, including diatomaceous earth or Kieselguhr, and in various states of activity, and is by far the most widely used adsorbent material. Various silicates are also used of which Florisil is the most popular. Alumina (aluminum oxides) is available in several forms, differing in surface activity and acidity. Other inorganic sorbents, such as activated carbon, metal oxides and clays are also available.

Organic sorbents include cellulose and its derivatives, starch, sugar, and dextran gels. These materials are more useful for the separation of hydrophilic substances, and thus are of special interest to the biochemist for application to materials such as amino acids, sugars, nucleic acid derivatives, etc. Although reverse phase C^{18} modified silica surfaces and polymer surfaces are commonly used for polar compounds, no specific references were found.

In actual practice, the thin layer chromatographic separation is conducted using layers of sorbent medium of about 25 micron thickness. Plates, usually glass, are 10 to 20 cm long and 5, 10 or 20 cm wide. Solutions containing the compounds to be separated are introduced by spotting or streaking on the plate, at a distance of about 1.5 cm from the lower edge. After the application solution has evaporated, the plate is placed in a developing chamber containing the mobile phase at a depth of about 0.5 to 1 cm. The developing solvent then moves upward by capillary action, passing over the applied material, and transporting individual components at various rates. After the solvent front has advanced some distance (10-20 cm), the plate is removed from the tank, and the solvent allowed to evaporate. The plate is then treated in some fashion to visualize or otherwise detect the chromatographed compounds.

Retention is usually expressed as a value (R_f) defined as the ratio of the distance of the transported compound from the point of application to the distance of the solvent front from the point of application. Because of variations in spot size, evenness of solvent flow and bed homogeneity, R_f values are often approximate. Common practice dictates the use of reference standards, chromatographed on the same plate, whenever possible.

Compound detection is accomplished by several procedures, the most direct being the use of UV light to visualize chromophoric substances. Adsorbents are available which contain an inert fluorescent reagent which is quenched by many compounds when viewed under UV light. Spots thus appear as dark regions against a bright, fluorescing background.

Many reagents are available for detection of specific types of compounds. These reagents are usually sprayed evenly over the TLC plate, which can lead directly to color forming reactions, can produce UV absorbing derivatives, or can form derivatives which can be further treated (e.g., heat) to render solutes as visible spots. Comprehensive lists of specific reagents and procedures are available from a number of sources (1,2). Quantitation procedures are discussed in detail by Touchstone (3).

TLC can be used for the separation and quantitation of complex mixtures, and has proven useful as a clean-up technique for analytes present in complex matrices such as biological fluid extracts or environmental samples.

Chemicals Determined

With the exception of some macromolecules, reactive compounds, and volatile substances, virtually any organic compound can be analyzed by TLC. Many inorganic ions can be analyzed as well. A scan of the applications of TLC in any comphrensive text (1,2) provides examples and full discussion of compound classes, including a wide variety of natural products (steroids, alkaloids, terpenoids, vitamins, etc.), drugs, pesticides, and dyes. Specifically, TLC has been applied to the analysis of metabolites of chlorinated pesticides in urine (7) and to the metabolites of toluene and styrene in urine (8).

Methods of Quantitation

Although TLC spot size can be related to the amount of substance producing the spot, direct measurement of the resulting spot is difficult and error-prone. Several means of quantitation of TLC components using spectroscopic methods exist, and have benefitted from continuing advances in appropriate instrumentation. These approaches, coupled with quantitation of radiolabelled substances on TLC plates, represent the most popular of current procedures. Other methods involve removal of spot components from the TLC plate and quantitatively analyzing the eluted species.

Scanning densitiometry is a technique whereby quantitative spectroscopic determination of a substance can be conducted directly on the thin layer chromatogram. Both UV absorption, and fluorescent emission or quenching can be measured during a continuous scan of the TLC plate, and the resulting photomultiplier signal can be electronically integrated. Densitiometers can be single beam or double beam, and can be operated in the reflectance or transmission mode. For direct transmission, the plate material must be sufficiently transparent to UV light at the wavelength chosen for quantitation. Although it has been shown (4) that, on theoretical grounds, quantitation by transmission is superior to reflectance methods, both can be utilized in routine practice, the mode selected often depending on other factors. Double beam instruments are usually more sensitive due to decreased baseline noise and drift problems.

The quantitation of TLC separated radiolabelled components can be accomplished by various means. The oldest and most sensitive technique involves removal of the radioactive zones from the TLC plate, and determining radioactivity by scintillation counting. This approach is tedious and time-consuming. A far simpler, though not very rapid means of quantitation of radiolabelled TLC components, consists of exposing a portion of X-ray film held in close contact with the plate. The film is developed after suitable exposure, usually one or more days. Quantitation is achieved using photodensitiometric methods.

The most expeditious means of quantitating radioactive TLC spots is through the use of a radioscanner, a device that contains a detector element (hot wire) which is sensitive to ionization produced by decay particles. The plate is either scanned by the detector, or exposed to a detector head that is of similar dimensions to the longitudinal axis of the plate. The voltage output (proportional to surface radioactivity) is recorded via a strip chart device. Microprocessor controlled units are available that incorporate integration algorithms in the operating software.

Limits of Detection

The wide variety of TLC detection modes, and the large number of different color reactions and individual compound responses, only permit general statements of detection limits. For visualization using color reactions or charring techniques, lower limits of detection are usually around the low microgram level, although nanograms levels of intensely colored species can be detected. For the measurement using densitiometry, UV absorbance, fluorescence emission, or fluorescence quenching, about 50 micrograms (7,8) of various species were quantifiable; certain highly fluorescent materials, such as quinine sulfate, possess a lower quantifiable limit of around 20 ng (3).

Radioscanners can detect increasingly lower levels of activity, as instrument sophistication is advanced. There is some difficulty in quoting limits of detection since a variable such as scan time can be altered, at the convenience of the operator, to maximize response. Modern scanners possess detection efficiencies approaching 100%. For 20 minute scans, about 250 dpm ^3H and about 50 dpm ^{14}C provide detectable signals.

Precision

Reproducibility of quantitative data can, for the reasons cited in the previous section, vary over a wide range of values. Privett et al. (5) in the determination of lipids via charring and densitiometry reported % relative standard deviation (%RSD) of about 2 to 3% for various lipid classes. Scanning densitiometry used in the determination of recovery of sugars from known sample mixtures gave %RSD of about 5% (6). Bieniek et al. (8) report %RSDs of 5 to 10% for the analysis of metabolites of toluene and styrene. These values are very acceptable, and result only when careful attention is paid to the method of application of sample to the plate.

Precision associated with determination of radioactivity via autoscanning is approximately on the level of the counting statistics of the instrument.

Accuracy

Mansfield (6) states that quantitative TLC can give results comparable in accuracy to other chromatographic methods. Using color development reactions to sugars, accuracy for visual estimation (with comparison to reference standards) was quoted as about ±20%. Colorimetric analysis of extracted TLC spots gave accuracies of ±10%, as did densitiometric techniques. A study of tritium: carbon 14 ratios for labelled steroids, using scintillation counting after two-dimensional TLC gave values within 97 to 100% of theoretical values. For radioscanning techniques, accuracy of about ±10% has been quoted by some manufacturers.

SAMPLE PREPARATION METHODS

Organic compounds of interest must be extracted from the sample and concentrated prior to TLC. Specific sample preparation methods are discussed in Section 11.

Simplicity, Cost and Sample Throughput

Thin layer chromatography can be accomplished with a minimum of expensive equipment, reagents and supplies. In terms of operation, it is one of the simplest chromatographic techniques. Although sophisticated instrumentation can be required for precise and accurate quantitative TLC analyses, routine screening methods employ readily available materials and require only modest laboratory skills. The ability to analyze several samples (10-15) on a single plate increases sample throughput. In a typical screening analysis, using two plates developed simultaneously, a single technician can apply, develop, and detect (spray) roughly 24 samples in one to two hours.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

Although sufficient information was not available from the references obtained to suggest a reliable method/analyte correlation parameter, TLC analysis is compatible with virtually all organic compounds with the exception of some macromolecules, reactive compounds and volatile substances.

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SECTION 16

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

INSTRUMENTATION

Principle of the Technique

Chromatography, discovered in 1903 by a Russian scientist Tswett (1), is a process in which components of a mixture are separated in a two-phase system. In liquid chromatography (LC), the stationary phase consists of either a solid or a liquid that is coated on or bonded to a solid. The moving, or mobile, phase is a liquid. Conditions are established under which the individual components flow at different rates under the influence of the mobile phase. The differing rates of elution occur because of interaction between the sample components and the stationary phase. There are four principal mechanisms in LC by which components of samples are selectively retained. These are (1) differences in partition coefficients (liquid-liquid chromatography), (2) differences in adsorption effects on surfaces such as silica gel (liquid-solid chromatography), (3) differences in dissociation of weak or strong electrolytes (ion-exchange chromatography), or (4) differences in molecular size or shape (steric exclusion chromatography). The mechanism resulting from differences in partition coefficients can be further subdivided into reversed-phase (RP) chromatography, in which hydrophobic interactions occur between the sample components and nonpolar groups bonded to the sorbent or support, and normal phase (NP) chromatography in which interactions occur with polar groups bonded to the sorbent.

Performance Parameters--

The interaction of a sample component with the stationary phase is referred to as retention and is quantified in liquid column chromatography as the retention volume (V_R), which is the volume (generally expressed in mL) of mobile phase which flows through the column system from the moment of sample introduction to the moment of appearance of the maximum concentration of the eluting peak at the detector. When a sample component does not interact at all with the stationary phase, it is said to be unretained and elutes in the void volume (V_0) or dead volume of the column. Since the retention volume is absolute only for a given sample component on a given chromatographic system, it is useful to express the retention of a sample component relative to the elution of a nonretained component. This is commonly done by means of the relative partition coefficient or capacity factor, k':

$$k' = (V_R - V_0)/V_0$$

Since chromatography is a separation method, one is always concerned with more than one component in a sample, and it is important to define the separating power of a column for the component of interest. Selectivity, α, is expressed as the ratio of the capacity factors of two components of interest:

$$\alpha = k'_v/k'_a$$

In practice, the selectivity factor, α, must be greater than 1 for separation to occur, and often lies in the region between 1.1 and 2.0. The selectivity factor alone does not indicate whether full resolution of the two components will be achieved. The individual component bands, or widths, must occupy a sufficiently small volume of the mobile phase so that the bands do not overlap. A sample is introduced rapidly at one end of the column as a concentrated band. As this band passes through the column, spreading occurs due to thermal motion of the molecules, the random and nonequilibrium character of the chromatographic process, and the existence of both longitudinal and transverse flow-rate profiles in the mobile phase. If the spreading rate of the bands of the components is less than the difference between the rates of their movement along the column, then these components can be separated.

The ability of a column to minimize band spreading is referred to as the efficiency of the column. The efficiency of all chromatographic processes is expressed quantitatively as the number of theoretical plates, N, of the column.

$$N = 16(t_R/W_{0.5})^2$$

where t_R is the retention time and $W_{0.5}$ is the width of the peak at one-half its height (expressed in units of time).

The resolving power, or resolution R, of the column for two components A and B, is calculated as follows:

$$R = 2(t_{Rb} - t_{Ra})/(W_b + W_a)$$

A resolution of 1.0 is achieved when the difference in retention time (or volume) between the maxima of peaks A and B is equal to half the sum of the widths of the bases of the constructed triangles, i.e., the adjacent triangles just touch at the baseline.

The reader may consult basic texts in chromatography (1,2) for a more detailed treatment of the above topics.

Instrumentation--

The minimum equipment required for LC experiments includes (a) a solvent reservoir, (b) at least one pump to propel solvent through the system, (c) a sample injector, (d) a column, where the actual separation occurs, (e) a detector, and (f), a recording device. For gradient elution analysis, a second pump, a solvent mixing tee and a pump controller are also required. Other possible components of an LC system include filters, pressure measurement devices, pulse dampeners, column thermostats, integrating recorders or computers.

The pump is one of the most important components in the LC system. It must be capable of delivering solvents accurately, precisely and pulse free at flow rates ranging from 0.5 to 2.0 mL/min for most applications (although some columns require flows as low as 10 microliters/min or as high as 30 mL/min). Several types of pumps are available including syringe-type, and single and double piston reciprocating pumps.

The sample injector is also of critical importance to the overall success of an LC analysis. A poor injection method can result in broad, poorly resolved peaks. Most LC systems today use one of the so-called loop injection valves. To use these injectors, the sample is loaded into the "loop" while the valve is in a position in which the mobile phase is bypassing the loop and passing directly to the column. To inject the sample, the valve position is changed resulting in the mobile phase passing through the sample loop and sweeping the sample onto the column. Both external and internal loop injectors are available which can precisely inject volumes ranging from 60 nL to 10 mL or more.

The column is the heart of the chromatographic system. For high performance liquid chromatography (HPLC), the column itself is generally made of stainless steel and is sometimes glass-lined. The standard-size, analytical columns have an external diameter of 1/4 in, and internal diameters of 2.6 to 5.0 mm.

Preparative columns have larger dimensions: 3/8 to 5/8 in. O.D. and 6.4 to 12.7 mm I.D. The recently introduced microbore columns have internal diameters of 1.0 mm. All of these column sizes are generally prepared in 25 to 30 cm lengths, although very short (about 5 cm) analytical columns are sometimes used for high-speed analyses. Packed analytical and microbore columns are seldom made longer than 30 cm due to difficulties in packing, however, multiple columns are sometimes coupled to provide higher separating power.

The choice of column packing depends on the compounds and samples being analyzed. In general, there are fewer column packings available in HPLC than in gas chromatography (GC). They cover a range of polarities from the very nonpolar octadecylsilane packing, which is generally used for the analysis of neutral to moderately polar sample components. It separates compounds via liquid-liquid partition and shows great retention for nonpolar compounds such as polynuclear aromatic hydrocarbons. At the other extreme is the nonbonded silica gel column. It separates components via the liquid-solid adsorption principle and is most often used for polar to moderately polar compounds. The reader is referred to a basic text for further information (1,2,3).

The final major component of the HPLC system is the detector. Requirements of a detector for HPLC are summarized below:

(a) The detector should be designed so that it does not contribute to band broadening in the system.

(b) It should be nonresponsive to the mobile phase and in particular should not respond to changes in flow rates, composition, or temperature.

(c) The detector should have high sensitivity with a wide linear dynamic range.

(d) It should either respond to all solutes, or it should have predictable specificity.

(e) It should have a fast response time in order to record fast peaks.

(f) It should be reliable and easy to operate.

Although no detector presently available provides for all of these requirements, many detectors are available, one of which is generally acceptable for a particular application. These detectors have been reviewed elsewhere (2,4,5), however, a summary of the most commonly used detectors follows.

The most widely used detector is the UV detector which measures the change in the UV absorption as the solute passes through a flow cell in a UV transparent solvent. Either a deuterium or mercury lamp is used to emit light in the UV range. The lamps can be used with or without a monochromator to provide, respectively, fixed or variable wavelength. The advantage of the variable wavelength detector is that solutes can be monitored at their wavelength of maximum absorption to optimize sensitivity. Current detectors are capable of detecting concentrations in the eluent of about 1 part in 10^9 molecules having high molar extinction coefficients. This corresponds to a few nanograms of injected material. If a molecule does not possess a sufficiently strong UV chromophore for satisfactory UV detection, derivatization may be used.

Refractive index (RI) detectors function by measuring the change in refractive index in the eluent as the solute flows through the sample cell. RI detection is much less sensitive than UV detection. Levels of about 1 microgram of material injected provide the detection limit of RI detectors. RI detectors do have the advantage that many UV nonabsorbing analytes can be measured directly without derivatization. Gradient elution is not a possibility with RI detectors, since the baseline continuously varies as the solvent composition changes.

Fluorimetric detectors function by exciting a solute with UV radiation at a given wavelength. The emitted fluorescent energy (at a longer wavelength) is then detected. Both the excitation and emission wavelengths can be varied to provide maximum sensitivity and selectivity. Fluorimetric detection can be performed both on compounds which naturally fluoresce and on compounds which have been derivatized. Fluorimetric detectors are theoretically more sensitive than UV detectors, although this increased sensitivity has only been recently accomplished with more sophisticated instrumentation.

Many applications of the use of electrochemical detectors have been reported in the literature. Electrochemical detectors provide sensitivity which is similar to that of UV detectors, however, they are frequently plagued by high background caused by solvent impurities, thus reducing their effective sensitivity. When using electrochemical detection, the chromatographic separation must be performed using aqueous or semiaqueous solvents since a high concentration of electrolyte is necessary for satisfactory detector operation.

This electrolyte may be added to the column effluent just prior to the detector if the separation is sensitive to the electrolyte concentrations.

The use of the mass spectrometer as a detector in HPLC has received much attention in recent years. The mass spectrometer has the marked advantages of universal response, high sensitivity and definitive identification. However, the task of interfacing mass spectrometry to HPLC is formidable because of the problem of transferring a liquid containing dissolved solids into a high vacuum chamber. Although LC/MS has become a reality in recent years, it is usually in the form of an expensive and sophisticated system.

Efforts have also been made to use Fourier transform infrared (FTIR) spectrometers as HPLC detectors, but this technique is still in the development stage.

Chemicals Determined

The HPLC is potentially applicable to virtually any organic compound, as well as certain ionic species, so long as correct choice of column and detector is made. Citations found in this review include analysis of chlorinated phenols (6), nitrophenols (7), benzidine and its derivatives (8,9), phthalates (10), amines (11), metabolites of toluene and xylene (12), many polynuclear aromatic hydrocarbons (12-15,23,24), amides (16), catechol (17), zinc (18), and oxalate ion (19,20).

Method of Quantitation

Quantitative analysis by HPLC relates detector signal (either peak height or area) to the concentration or total amount of analyte injected onto the column. Both internal and external calibration methods are used. For the external standard method, calibration solutions of the components of interest are prepared in the same solvent as the sample. These calibration solutions are injected onto the LC and the detector response (i.e., peak area) is measured for these known concentration of the components of interest. Concentration in an unknown sample is then determined by ratioing the detector response for the sample against the detector response for the standard.

For the internal standard technique, a calibration standard is prepared which contains an internal standard at a known concentration. The same internal

This electrolyte may be added to the column effluent just prior to the detector if the separation is sensitive to the electrolyte concentrations.

The use of the mass spectrometer as a detector in HPLC has received much attention in recent years. The mass spectrometer has the marked advantages of universal response, high sensitivity and definitive identification. However, the task of interfacing mass spectrometry to HPLC is formidable because of the problem of transferring a liquid containing dissolved solids into a high vacuum chamber. Although LC/MS has become a reality in recent years, it is usually in the form of an expensive and sophisticated system.

Efforts have also been made to use Fourier transform infrared (FTIR) spectrometers as HPLC detectors, but this technique is still in the development stage.

Chemicals Analyzed

The HPLC is potentially applicable to virtually any organic compound, as well as certain ionic species, so long as correct choice of column and detector is made. Citations found in this review include analysis of chlorinated phenols (6), nitrophenols (7), benzidine and its derivatives (8,9), phthalates (10), amines (11), metabolites of toluene and xylene (12), many polynuclear aromatic hydrocarbons (12-15,23,24), amides (16), catechol (17), zinc (18), and oxalate ion (19,20).

Method of Quantitation

Quantitative analysis by HPLC relates detector signal (either peak height or area) to the concentration or total amount of analyte injected onto the column. Both internal and external calibration methods are used. For the external standard method, calibration solutions of the components of interest are prepared in the same solvent as the sample. These calibration solutions are injected onto the LC and the detector response (i.e., peak area) is measured for these known concentration of the components of interest. Concentration in an unknown sample is then determined by ratioing the detector response for the sample against the detector response for the standard.

For the internal standard technique, a calibration standard is prepared which contains an internal standard at a known concentration. The same internal metabolites from urine (12), 98 to 99% recovery for diethylamine (a metabolite of disulfiram) in urine (11), 98.6% recovery for zinc from blood (18), 99.1 to 100.2% recovery of oxalate ion from urine (19,20), and 83.4 to 95.4% recovery of various phthalates from urine (10).

Simplicity, Cost and Sample Throughput

High performance liquid chromatography is basically a simple and standard technique for the determination of a wide variety of organic compounds. Commercial instrumentation, including columns, pumps, detector, injection system, and data processing are readily available. Costs for a complete HPLC system range from about $6,000 for a basic system to $60,000 for systems including automation and full data processing. Sample throughput varies widely from as high as 20 samples/hour to less than one sample/hour depending upon the matrix, the compounds of interest and the resolution required. Labor for sample preparation costs can, in many cases, exceed the cost of the actual analysis.

SAMPLE PREPARATION METHODS

See Section 11.

RECOMMENDED METHOD/ANALYTE CORRELATION PARAMETERS

HPLC is potentially applicable to virtually any organic compound, as well as certain ionic species, so long as the correct choice of column and detector is made. It is particularly useful for those organic compounds which are too polar or insufficiently volatile for successful gas chromato-graphic analysis. HPLC is an excellent separation technique, however, it is sometimes difficult to identify unknown compounds in a mixture.

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SECTION 17

BODY BURDEN

Biological monitoring of humans for xenobiotic body burden is the collection and analysis of biological specimens (organs, tissues, blood, etc.) for the purpose of obtaining a chemical level of exposure to xenobiotics.

Biological monitoring is useful for several reasons. First and foremost, it provides direct evidence of exposure and absorption of a xenobiotic. It thus alerts one to the possibility of a human health hazard. The data obtained by biological monitoring can be correlated with the observed level of pollutants in the environment, as well as correlated with the incidence of human diseases which may be due in part to the presence of the pollutants. These correlations can be used to determine priorities with regard to research relating to human health, and to determine the necessity of regulation of pollution sources. Biological monitoring can point out pollutant problems which are not otherwise noted, such as could occur due to bioaccumulation of pollutants in humans resulting from long-term exposure to very low levels of pollutants in the environment.

In 1977 an international workshop was held in the Luxemborg on "The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants" (1). The objectives of the workshop were:

(a) To assess the types of environmental pollutants and human specimens most suitable for biological monitoring and to evaluate the probable usefulness of biological specimen banking.

(b) To examine the state of the art and technical feasibility of programs designed to collect human biological specimens for biological monitoring and biological specimen banking.

(c) To develop guidelines on sampling, sample preparation, storage, and analytical requirements.

(d) To draw up recommendations for further research and development.

Some of the results of the workshop (1) are summarized in Table 17-1.

The purpose of this section is to review the literature on body burden, in order to define which biological matrices are suitable indicators of environmental exposure. Table 17-2 summarizes the information from the data base "Chemicals Identified in Human Biological Medium" and Chemical Abstract Data Base relating target chemicals to biological matrix. Essentially, it shows the biological matrices in which the target chemicals were identified. Although this is useful information, it does not suggest which matrices will serve as the best indicator of long- or short-term environmental exposure to target chemicals. The following section will address this question with the discussion organized according to chemical class. Only citations which have related exposure to a biological matrix will be discussed. A compilation of body burden data for trace elements was published by Igengar et al. (2).

ORGANIC COMPOUNDS

Exposure routes for organic chemicals are usually inhalation, ingestion, and dermal absorption. Once in the body, the chemical may be excreted, (including exhalation) stored, or metabolized. In general, chemicals are metabolized to a polar form to allow excretion by the kidney. The fate of any chemical will depend upon its volatility, polarity, and chemical and biological stability. For example, polychlorinated biphenyls (PCB) and polychlorinated terphenyls (PCT) are nonvolatile, inert, lipophilic compounds which are stored in the adipose tissue. More polar compounds such as phenols and acids are generally excreted in the urine.

Carboxylic Acids

Absorption and urinary excretion of 2,4-dichlorophenoxyacetic acids (2,4-D) were studied in six subjects following oral ingestion (5 mg/km) (3). Results showed that 2,4-D is quickly absorbed with significant quantities detected in the plasma 1 hour after ingestion. The highest concentration (40 μg/mg in plasma) was reached in 7-24 hours followed by a steady decline in 2,4-D levels. More than 75% of the compound was found unchanged in urine after 96 hours. No metabolites were detected in any of the urine samples.

TABLE 17-1. RECOMMENDED TISSUES FOR MONITORING SOME EXAMPLE XENOBIOTICS

Tissues	Pollutants																	
	As	Br	Cd	Cr	F	Pb	Hg	Mo	Mn	Vi	V	Zn	Sr	Cu	Co	Pt/Pd	Sn	Methyl Hg
Adipose tissue																		
Blood	+	?	+	?	?	+	?	?	?	?	?	?	?	?	?	?	?	+
Hair and nails	+		?	?	?	+	?	?	?	?	?	?	?	0			?	+
Heart	?		?			?						?			?			?
Kidney	?		+	?	?	+	+	?	?	?	?	?		?	?	?		?
Liver	?		+	?	?	?	?	?	?	?	?	?	?	?	?	?	?	+
Muscles			0									?						?
Skin	?											?						?
Urine	+	?	+	+	+	+	+	?	?	+	?	?	?	?	?			0

(continued)

TABLE 17-1. (continued)

Tissues	Pollutants																							
	DDT organochlorine pesticides excluding phenoxyherbicides and pentachlorophenol	Phenoxy herbicides	Pentachlorphenol	PCB, PBB, PCH	Tetrachloro-dioxin benzofurane and azobenzene	Chlorinated solvents and plastic monomers	Fluorinated propellants	Nonsubstituted aliphatic aromatic volatile hydrocarbons	Polycyclic hydrocarbons	Amino and nitro derivatives	Nitroso derivatives	Alcohols (including long chain)	Glycols	Ethers	Ketones	Aldehydes, Acids, Amides	Phenol derivatives	Organo phosphorous esters	Phthalate esters	Carbon monoxide	Cyanide nitrile	Mycotoxins	Antibiotics	Hormonal substances
Adipose tissue	+		?	+	?	?	?	?	?	?		?							?	0	0	?		?
Blood	+	?	?	?	?	+	+	+	?	?	?	+	?	?	?	?	?	+	?	+	?	?	?	?
Hair and nails								0	0	0		0	0	0	0	0				0	0			
Heart																				0	0			
Kidney											?									0	0	?		
Liver	?	?	?	?	?	?	?	?	?		?						?		?	0	0	?	?	?
Muscles																				0	0		?	?
Skin				?	?							0	0							0	0			
Urine	?	+	+			+		+	?	?	?	+	?	?	?	?	?	?	?	0	?	?	?	?

$^{+}$Correlation with body burden.

$^{?}$Possibly useful as indicator specimen, but further research is required to confirm.

^{0}Known as not useful.

Blank = no information available.

TABLE 17-2. COMPOUNDS IDENTIFIED IN HUMAN BIOLOGICAL MEDIA

Compounds	Biological Media Adipose	 Blood	 Urine	 Skin	 Hair	 Nail
Organic Acids						
Cacodylic acid		X				
Trichloroacetic acid		X	X			
Chloroacetic acid		X	X			
2,4,5-Trichlorophenoxy acetic acid		X				
Oxalic acid			X			
2-Methoxy-3,6-dichloro-benzoic acid		X				
4-Amino-3,5,6-trichloro-picolinic acid		X	X			
Propionic acid			X			
Amines						
Benzidine			X			
3,3'-Dichlorobenzidine			X			
Ammonia		X				
o-Toluidine		X				
m-Toluidine		X				
p-Toluidine		X				
Tocainide		X				
Dimethylamine		X	X			
2,4-Diaminoanisole sulfate			X			
2,5-Diaminoanisole sulfate			X			
2,6-Diaminoanisole sulfate			X			
3,3'-Dimethoxybenzidine			X			
Nitrosoamines						
Nitrosopyrrolidine		X				
N-Nitrosodimethylamine		X	X			
N-Nitrosodiethylamine		X				
N-Nitrosodi-n-butylamine		X	X			
N-Nitrosopiperidine		X				
Phenols						
m-Catechol		X				
o-Resorcinol		X	X			
2,3,4,6-Tetrachlorophenol	X	X				
2,3,4,5-Tetrachlorophenol	X	X				
Phenol		X	X			
Cresol		X	X			
4-Chlorophenol		X				
2-Chlorophenol		X				
3-Chlorophenol		X				
2,4-Dichlorophenol	X	X				

(continued

TABLE 17-2. (continued)

	Biological Media					
Compounds	Adipose	Blood	Urine	Skin	Hair	Nail
Phenols (continued)						
2,4,6-Trichlorophenol	X	X				
2,4,5-Trichlorophenol	X	X	X			
Pentachlorophenol	X	X	X			
2-Nitrophenol		X	X			
4-Nitrophenol		X	X			
Miscellaneous Nitrogen-Containing Compounds						
Captan	X	X				
Dioxacarb	X	X				
Carbaryl	X	X				
Alcohols						
2-Propanol		X	X			
Methanol		X				
Ethylene glycol			X			
t-butyl alcohol			X			
Isobutyl alcohol		X				
Trichloroethanol		X				
Aldehydes						
Acetaldehyde		X				
Phosphate Esters						
Parathion		X	X			
Malathion		X				
Methyl parathion		X	X			
o,o-Diethyl phosphorodithioate		X				
Dichlorvos	X					
Dimethyl phosphorothioic acid			X			
0,0-Dimethyl phosphoro dithioate		X	X			
0,0-Dimethyl phosphorothioate		X	X			
Sulfate Esters						
Endosulfan		X	X			
Endosulfan sulfate		X	X			
Carboxylic Acid Esters						
Di-n-butylphthalate	X	X				
Di(2-ethylhexyl)phthalate	X	X				
Ketones						
Methyl isobutyl ketone		X	X			

(continued)

TABLE 17-2. (continued)

	Biological Media					
Compounds	Adipose	Blood	Urine	Skin	Hair	Nail
Halogenated Aliphatic Hydrocarbons (continued)						
γ-Hexachlorocyclohexane (Lindane)	X					
trans-Nonachlor	X					
1,1,2,2-Tetrachloroethene		X				
1,2-Dibromoethane		X				
Ethylene dibromide		X				
Fluorocarbons			X			
Polynuclear Aromatic Hydrocarbons (PNA)						
1,1-Biphenyl	X					
Anthracene	X					
Pyrene	X					
Benzo[e]pyrene	X					
Benzo[a]anthracene	X					
Benzo[b]fluoranthene	X					
Benzo[k]fluoranthene	X					
Benzo[a]pyrene	X					
Benzo[ghi]perylene	X					
Dibenzo[ah]anthracene	X					
Hydrocarbons						
m-Xylene	X					
p-Xylene		X				
Ethylbenzene	X	X				
Styrene	X	X	X			
Toluene	X	X				
n-Hexane			X			
Cyclohexane			X			
Metals						
Aluminum salts		X	X		X	
Antimony		X	X	X	X	X
Arsenic	X	X	X	X	X	X
Barium					X	
Cadmium	X	X	X	X		X
Calcium		X				
Chromium	X	X	X	X	X	X
Copper	X	X	X	X	X	X
Lead		X	X			
Manganese	X	X	X	X	X	X
Magnesium		X		X	X	X

(continued)

TABLE 17-2. (continued)

Compounds	Biological Media					
	Adipose	Blood	Urine	Skin	Hair	Nail
Ethers						
Dieldrin	X					
p-Dioxane		X	X	X		
Heptachloro epoxide	X	X	X			
Endrin		X				
Octachloro epoxide	X	X				
Nitriles						
Hydrogen cyanide		X				
Sulfur Compounds						
Carbon disulfide		X	X			
Dimethyl disulfide		X	X			
Halogenated Aromatic Hydrocarbons						
PCBs	X	X		X	X	
p,*p*'-DDT	X	X	X			
o,*p*'-DDT	X	X	X			
p,*p*'-DDE	X	X	X			
p,*p*'-DDD	X	X	X			
Hexachlorobenzene	X	X				
Chlorobenzene	X	X				
o-Dichlorobenzene	X	X			X	
m-Dichlorobenzene	X	X				
p-Dichlorobenzene	X	X				
Tetrachlorodibenzo-p-dioxin		X				
Halogenated Aliphatic Hydrocarbons						
Chlordane	X					
Aldrin	X					
Tetrachloromethane		X	X			
Dichlorodifluoromethane		X				
Trichlorofluoromethane		X				
Dibromochloromethane	X					
Bromodichloromethane		X	X			
Chloroform		X				
Dichloromethane		X				
Tetrachloroethene	X	X	X			
Trichloroethene	X	X	X			
Trichlorotrifluoroethane	X	X	X			
1,1-Dichloroethene		X				
1,2-Trans-vinylidene chloride		X				
Vinyl chloride		X				
α-Hexachlorocyclohexane	X					
β-Hexachlorocyclohexane	X					
δ-Hexachlorocyclohexane	X					

(continued)

TABLE 17-2. (continued)

	Biological Media					
Compounds	Adipose	Blood	Urine	Skin	Hair	Nail
Metals (continued)						
Mercury		X	X	X	X	X
Molybdenum		X		X	X	X
Nickel			X			
Palladium	X	X	X	X		
Silver	X	X	X		X	
Sodium		X		X	X	
Selenium		X	X	X	X	X
Strontium					X	
Tin					X	
Titanium		X	X	X	X	
Tungsten				X	X	
Vanadium	X	X	X		X	X
Zinc	X	X	X	X	X	X

These data suggest, that either plasma or urine may be used to monitor short-term exposure to 2,4-D and other similar compounds. Since the acid does not appear to accumulate in the body, measure of long term exposure may not be possible.

Nitrosoamines

There have been several reports of the presence of N-nitroso compounds in the human body. Fine et al. (4,5) detected volatile nitrosoamines in the blood and reported that the levels in blood increased after ingestion of a meal. Nitrosoamines have also been detected in human urine (6). Yamamoto et al. (7) detected nitrosodimethylamine in blood samples before and after eating. This study showed no correlation with ingestion of foods high in nitrate or secondary amines.

No studies have been performed to directly relate environmental exposure of nitrosoamines to body burden. The suggestion has been made that nitrosoamines will form *in vivo* after ingestion of nitrates and secondary amines, although these data are not conclusive.

Phenols

Pentachlorophenol (PCP) residues have been identified in human blood, urine, and adipose tissues. Test conducted by the Center for Disease Control showed that 34 residents of Kentucky homes made of logs treated with pentachlorophenol had mean blood PCP serum levels (∿1 ppm) seven times and mean urine (2-87 ppb) levels ten times those of individuals living in conventional homes (8). A study in Florida showed no relationship between drinking water PCP levels and blood PCP concentrations (9). Although other chlorinated phenols were included in the study, none were detected. Mean PCP concentrations in seven samples were higher for Hawaiian residents than other reported data (10). Since PCP is used widely as a pesticide in Hawaii, this data suggests analyses of serum samples may be appropriate for determining long term exposure to PCP.

Residue levels of PCP (∿20 pbb) in adipose tissues have been reported in several studies (9,11,12). Comparing these levels to residue levels of DDT and DDE in adipose samples suggests that PCP does not bioaccumulate to the same extent.

Hexachlorophene is absorbed directly through the skin after washings. Data show a direct correlation with washing and blood concentrations (13,14). During an eight week test, blood concentrations (~60 ng/mL) reached a peak during the third, fourth, and fifth weeks of usage. Mean blood levels returned to normal (<5 ng/mL) within two weeks after discontinuing washing (13).

Alcohols

Assessment of occupational exposure to methanol has been based upon the measurement of methanol itself (15-18) or its metabolites, i.e., formaldehyde (20) and formic acid (18-20) in blood and urine.

There is evidence that the level of formic acid in urine remains practically unchanged (21) after exposure to adequate concentrations of methanol vapors or that it increases so slightly that the increase can be proved only by a statistical analysis of a large number of results (20,21,23). For this reason, no correlation can be found between methanol concentration in air and formic acid levels in blood and urine (20).

Experimental evidences has shown that it is better to monitor the unchanged alcohol in blood and urine. Leaf and Zatmal (17) demonstrated that methanol concentrations in urine are directly proportional to blood concentration.

In another study, five volunteers were exposed to constant concentrations of methanol vapors for 8 hours (21). The retention of methanol in the lung and the course of excretion in urine were monitored. Lung methanol levels did correlate well with whole shift urine measurements.

Phosphate Esters

Exposure to organophosphate pesticides is routinely determined by measuring metabolites in urine. *p*-Nitrophenol (PNP) has been used successfully as an indicator of exposure to methyl Parathion, ethyl Parathion, and ethyl *p*-nitrophenyl phenylphosphonothioate (22-24). Alkyl phosphates such as dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethylthiophosphate (DETP), and diethyl dithiophosphate (DEDTP) have also been used to monitor methyl and ethyl Parathion, malathion and Gluthion exposure (22,25,26).

One study demonstrated rapid excretion of p-nitrophenol and diethyl thiophosphate, while DMP and DEP were excreted more slowly (22). Cumulative excretion of PNP and the alkylphosphate gave a close dependence curve to Parathion insecticides.

In another study, twelve cotton insect scouts were monitored during an entire pesticide application season by weekly determinations of urinary PNP. The maximum mean amount of methyl and ethyl parathion absorbed by calculation from PNP excretion in their urine was 0.95 mg/24 h (23).

Esters

Analysis of blood samples before and after meals was performed to demonstrate an increase in di-n-butyl (DNBP) and di(2-ethylhexyl)phthalate (DEHP) levels resulting from contaminated food (27). The average values measured after eating (0.10 DNBP and 0.13 DEHP) are reasonable if 1 kg of food containing 1 ppm DNBP or DEHP was consumed by an individual of 60 kg body weight and 40% of the plastizer ester appeared in the blood in 2 hours after ingestion. Studies with rats have shown that more than 80% of administered DNBP and DEHP is excreted in the urine of rats that received a single low dose orally (28,29). Consequently, either blood or urine should be good matrices for assessing environmental exposure.

Ketones

Experiments with radiolabelled methyl n-butyl ketone (MBK) on human subjects demonstrated efficient absorption in the lungs, the gastrointestinal tract and through the skin (30). About 5% of the oral dose was excreted in 8 days. Only a small portion of the radioactivity was also excreted in the urine. Although the urinary radioactivity was not characterized, previous studies in rats showed that MBK was excreted in urine as 2-hexanol and 2,5-hexanedione (21).

Organochlorine Pesticides

Exposure to organochlorine pesticides may be monitored using either blood or adipose tissue. Because of their hydrophobic nature, these compounds are not normally found in urine samples. Blood is most frequently used to assess exposure since it is easier to obtain than adipose samples.

Several researchers have shown high concentration of DDT (33-35), DDE (33-35) and HCB (35) in plasma from exposed workers compared to a nonexposed control population. Although no significant correlation was observed between the total DDT equivalent in serum and duration of occupational exposure (31).

Davis (36) determined p,p'-DDT and p,p'-DDE concentrations in blood from 84 occupationally exposed individuals in south Florida. They found DDT levels were transient and related to recent exposure of workers.

A study by Burns et al. (37), demonstrated that blood concentration of hexachlorobenzene, hexachlorobutadiene and organochlorine pesticides were related to work exposure. In this study, blood levels also correlated with dust levels in the working environment. On the other hand, Dale (38) was unable to define a quantifiable relationship between blood and dust levels for organochlorine pesticides, but the data did show a trend. Palmer (39) reported that Lindane concentrations in plasma corresponded to the rated degree of exposure. Likewise, mean blood Lindane concentrations were demonstrated to increase after topical application of Lindane with a maximum concentration reported at six hours (40). Bristol et al. (170) found no significant increase in 25 specific nonvolative organochlorine contaminates in the blood of potentially exposed residents of Love Canal.

Mahmood and Al-Shahristani studied the exposure of newborn infants to DDT (183) by determining the levels of DDT in maternal blood, infant blood, and mother's milk. They found the concentration of DDT in milk to be 5.7 times higher than in maternal blood. At the same time, the concentration in newborn infant's blood was found to be only one-third that in the blood of their mothers indicating that the placental barrier hinders the transfer of DDT and DDE from mother to fetus.

Nishimura et al. (184) and Unger and Nordberg (185) have studied the distribution of DDT, DDE, β-BHC and PCBs in various human tissues. Nishimura et al. (184) examined cerebrum, heart, liver, kidney, and skin levels in human embryos and fetuses. Their results indicated that the concentrations of PCBs, p,p'-DDE and β-BHC were highest in skin and lowest in the brain. Unger and Nordberg (185) studied fat, basal ganglia, spleen, liver, kidney, ovary, and testis for PCBs and DDT-related compounds and found that these compounds accumulate mostly in fat; the concentration in fat were an order of magnitude greater than in other tissues. The next greatest levels were seen in the liver with only 6 to 10% as much as in fat.

PCB concentrations in blood have also be correlated with environmental exposure. Blood PCB levels in breast fed babies were elevated compared to a nonoccupationally exposed control population (41). Chen et al., have shown differences in PCB patterns and concentrations for individuals from different regions and different occupations. Elevated blood PCB levels were reported for Yusho patients five years after exposure (42) and even at this time, a positive correlation exists between the total amount of rice oil consumed and PCB level (43).

Most of the organochlorine pesticides and PCBs bioaccumulated in adipose tissue due to their chemical and biostability as well as their lipophilicity (44). Reported concentrations of DDT and its metabolites in adipose are approximately 50 to 100 times greater than in blood for the same person (45). In another study, concentration of Dieldrin in an adipose sample of a pesticide worker was approximately 100 times greater than in a nonoccupationally exposed worker (46).

Hattula et al. were able to correlate measured PCB and DDT adipose concentration with age, sex, and geographic location employing differences in exposure level (47). Several studies have been conducted to determine concentrations of PCBs, PCTs, PCDFs, and PCQs (48-50,171) in human adipose samples. Results showed very high levels of PCBs and PCTs in individuals exposed from ingesting contaminated rice oil compared to an unexposed population. The PCB and PCT pattern in adipose from rice oil exposure was significantly different from that obtained from exposed workers. The high concentration of PCTs and PCDFs in adipose tissue compared to PCBs was interesting since exposure to PCTs should have been low in all cases. Apparently, the PCTs have a higher affinity for bioaccumulation. Most of the PCDF isomers decreased in liver after 18 months, but those that were retained had two vicinal unchlorinated carbon atoms in at least one of the two aromatic rings.

Halogenated Aliphatics

Absorption, excretion, and storage of volatile halogenated organics has been studied by a number of researchers.

Studies monitoring human exposure to controlled vapor phase concentrations of radiolabelled methylene chloride have shown that as much as 90% of the inhaled vapor is absorbed by the pulmonary route (51). During exposure, the uptake of CH_2Cl_2 in the blood was gradual and again directly proportional to the magnitude of exposure. Within 6 hours blood CH_2Cl_2 levels had returned to normal. Blood concentrations ranged from 0.1 to 2 ppm for similar exposure levels. Blood concentrations for an accidental poisoning were ~500 ppm.

Muller et al. (54-57) showed that after trichloroethylene inhalation, the metabolite trichloroethanol (TCE) is excreted rapidly in the urine, while trichloroacetic acid (TCA) accumulates in the organism due to high plasma protein binding (54). Concentrations of 5 ppm TCA and 2.3 ppm TCE were found in plasma after a 5 day exposure period. This author suggests monitoring trichloroethylene exposure by measuring both metabolites in the plasma. Wilson and Ottley (172) followed the elimination of 1,1,1-trichloroethane from the body via the breath of an exposed individual.

The reported metabolic half-lives for perchloroethylene (PCE) and trichloroethylene (TCE) are 144 and 41 hours respectively for urinary metabolites (59). These half-lives are many times longer than similar determinaton for the nonhalogenated hydrocarbons toluene, xylene, styrene, and phenol (60,61).

Vinyl chloride (VC) exposure has been associated with excess cancer incidence among vinyl chloride-exposed workers, and in animals. In laboratory animals most of the VC adsorbed during inhalation was metabolized. Of a radiolabelled dose, only 0.02% was detected as expired monomer, with 98% of the total recovered. A small amount of radioactivity retained after 75 hours, mainly in the liver. Vinyl chloride has been detected in the subcutaneous fat of polymerization workers. In light of its volatility and its facile metabolism in rates, the lipophilicity of VC appears to aid fat retention in humans (62).

Total organic fluorine in blood has been used to monitor exposure to fluorochemicals in the work environment. Fluorine levels in workers decreased as exposure sources were removed (61).

Aromatic Hydrocarbons

Human uptake and metabolism of styrene has been fairly well studied. Styrene can be absorbed by inhalation or skin contact. During exposure to

20-100 ppm, about 60% of the styrene inhaled is absorbed by the body (62). Of this amount, metabolism to mandelic (85%) and phenylglyoxylic acid (10%) accounts for most of the absorbed styrene. Metabolism to hippuric acid and conjugates of phenyl glycol also occurs (60).

Styrene is very soluble in blood and fat, more than many halogenated hydrocarbons. Following exposure at 100 ppm, blood levels of 0.2-15 ppm styrene have been determined (63,64). As with PCE and TCE, blood levels are proportional to the intensity, and not duration, of exposure.

The urinary half-life of styrene metabolites is about 8 hours, with detectable levels continuing for 30 hours.

Styrene and ethyl benzene have been measured in the subcutaneous fat of workers with levels of 0.2-1 ppm roughly related to the degree of exposure and time since last exposure.

The blood levels of these workers were measured separately and were in the 0-10 ppb range.

Fat levels of styrene were detected more than 48 hours following the last exposure, longer than detectability in breath of experimental subjects after sustained exposure at 100 ppm (64). This finding is of interest, since styrene is a monomer more readily metabolized than the halogenated hydrocarbons. Engström et al. (173) studied the uptake, distribution, and elimination of styrene from subcutaneous adipose tissue in man. They estimated the half-life in adipose to be two to four days.

Exposure to other aromatic hydrocarbons has been measured either by directly measuring the compound in blood or by measuring a metabolite in urine. Kometzko et al. (66) were able to show a 10 to 20-fold increase in blood toluene levels under irregular, long term, exposure conditions. Likewise, Savolainen et al. (67) were able to show a direct relationship between xylene inhalation and blood concentration.

Engström and Riihimäki studied the distribution of m-xylene to subcutaneous adipose tissue in short-term experimental human exposure (174). 3.7 to 8.0% of the total body uptake was estimated to distribute to adipose tissue. The median elimination half-time of m-xylene from this tissue was 58 hours.

In another study, the relationship between the solvents ethylbenzene and xylene in blood and the excretion of phenolic compounds in urine was tested (68). It was shown that approximately 1% of retained ethylbenzene is metabolized to 2-ethylphenol. Other metabolites include acids. There was a direct linear correlation between ethylbenzene in blood and ethyl phenol in urine. Finally, Van Haaften et al. (68) were able to show a direct relationship between benzene exposure and phenol concentration in urine.

Polynuclear Aromatic Hydrocarbons

The in vivo fate of polynuclear aromatic hydrocarbons (PAHs) is of great interest because of the known mutagenic and carcinogenic activity of many classes of these compounds. Under environmental conditions, PAHs generally enter the body via inhalation of particles onto which the compounds are adsorbed. Although the clearance of large particles (15 to 30 μm) from the lung occurs in several days, smaller particles (0.5 to 1.0 μm) display a longer residence time (175,176). It is from these smaller particles that PAHs can desorb to some extent and react with respiratory tissue (175). The fact that a great number of the particles are cleared does not mean that the organism is free of danger since most of these cleared particles are swallowed. PAHs, e.g., benz[a]pyrene, can be subsequently absorbed through the gut (177).

Regardless of the route of administration, PAHs, once absorbed, become localized in a variety of body tissues. The distribution of radioactivity derived from ^{14}C-BaP in the rat and mouse was determined following subcutaneous, intravenous, and intratracheal administration (178). Blood levels of BaP following intravenous injection reached a maximum very quickly, but radioactivity became barely detectable after ten minutes. Concentrations in the liver reached a maximum within ten minutes after injection and corresponded to 12% of the total dose. Radioactivity in the liver was reduced to 1 to 3% of the administered dose within 24 hours. Maximum radioactivity derived from labeled BaP was recovered in the bile and feces. However, a very large percentage (~40%) was associated with intestinal tissue (not feces) 24 hours after intravenous injection of an aqueous suspension. It was later shown that unmetabolized BaP was extensively located in the mammary gland and general body fat after a single feeding of 10 to 30 mg of the carcinogen (179). The effect of molecular structure on PAH localization in mammary glad and fat has been shown to be quite dramatic (179).

The tissue distribution of ^{3}H-dibenzo[g,c]carbazole following intratracheal instillation was qualitatively and quantitatively similar to BaP; the greatest amount of radioactivity was localized in the intestines of treated hamsters (180).

It has long been recognized that various PNAs are primarily excreted through the hepatobiliary system and the feces (178,181,182). Consequently, PAHs do not accumulate to the same extent as organochlorine pesticides (e.g., DDT). This does not, however, make the effects of PAHs, either as parent or metabolite, any less deleterious to an organism.

ELEMENTS

Several reports found in the published literature, described the simultaneous analysis of many elements in human biological matrices.

Significant differences in the trace levels of chlorine, potassium, calcium, titanium, manganese, iron, copper and lead in hair from different parts of the body were found (70). This suggested that the trace elemental absorption and accumulation in hair not only depends on the particular element, but also on the location of the hair in the body. The trace concentrations in white and black hair was also found different.

Seventeen different elements were analyzed simultaneously in healthy and pathological tissue for the purpose of obtaining information on the cancerous process (71). Significant differences in the content of various elements were found in the normal and pathological tissue. Potassium, zinc and selenium were found in higher concentration in cancerous mucosa of the stomach. No significant differences appeared in the elemental composition of blood, erythrocytes, hair and striated muscle taken from the abdominal wall from patients with gall bladder or stomach cancer as compared with other noncancer diseases.

Human hair root has been analyzed for the presence of several elements (72). Hair root, rather than strand may reflect the most recent nutritional influences and its analysis is exclusive of externally acquired constituents. Samples collected from 23 randomly selected rural Florida children, three to six years of age were analyzed. The results showed differences in the Fe content by sex and Cu (and possibly others) differences caused by intake.

Human blood serum was analyzed for zinc, copper, iron, chromium, manganese and selenium as part of a survey to determine whether or not the Australian aboriginal people received optimal diet (73). Special attention was directed to chromium because of the high incidence of diabetes mellitus in this population.

Multiple element analysis of human cerebrospinal fluid and other tissues was used to determine diseases of the nervous system (74). The distribution of copper, iron and zinc in the cerebrospinal fluid was such that deficiencies could not be studied. For the majority of trace elements studied, the normal values could not be determined, much less the deficiencies. The interpretation of sporadic high levels was not possible. High levels of silicon found was not anticipated. The accumulation of silicon in the body fluids of patients with renal failure and on dialysis coupled with the clinical correlations made silicon a candidate for a uremic neurotoxin. The higher levels of silicon in cerebrospinal fluid of clinically diagnosted and autopsy proven cases of Alzeheimer's disease coupled with the presence of silicon in the neurofibrillary tissue of these patients argues for a correlation with this poorly understood disease.

Twenty-four elements were measured in hair from 20 individuals who worked in a lead-zinc smelter in Poland by Tomza et al. (186). These same elements were also measured in hair taken from 20 individuals considered "normal" or not exposed. Elements found to be at elevated levels relative to the controls included As, Se, Ag, Cd, and Sb.

Arsenic

Urinary levels of arsenic have been used to monitor worker exposure to inorganic arsenic. Generally, determination of methylated forms of arsenic indicate arsenic contribution due to diet. The proportion of methylated arsenic species to inorganic forms gives information concerning the length of time between exposure and sampling (75).

A study was carried out on children living in smelter communities. Urinary arsenic levels in these subjects were higher than nonexposed children living in rural areas. Speciation shows that this increase was not due to organoarsenicals in food (76). Increased arsenic levels in urine were shown to be related to arsenic concentrations in drinking water and airborne arsenic levels (77).

Hair, blood, and urine analysis for arsenic content in samples collected from young boys indicated that the best matrix to monitor nonoccupational exposure is hair (78). Blood aresenic did not correlate with exposure to arsenic in water. Arsenic in hair and urine did correspond to arsenic exposure (79).

Human subjects were fed cacodylic acid; their blood and urine were then collected and analyzed for arsenic content. Urine concentrations checked well with arsenic intake; blood correlated poorly (80).

Another feeding study involved the ingestion of sodium metaarsenite by volunteers. Urine was collected and arsenic levels measured as total inorganic, monomethylarsenic acid, and dimethylarsenic acid. Urinary arsenic determinations were proposed as a means for the biological testing of exposure to arsenic (81).

Urine, hair, and nail samples were analyzed for arsenic content and the correlation tested with levels in water. Urinary arsenic measurements correlated better than hair levels (82). Arsenic in hair was shown to be a good indicator for long-term exposure (83).

Arsenic levels in human tissue and milk from subjects who had or were living in the vicinity of Bombay, India, were measured by Dang et al. (190). The levels found in the tissue samples were high relative to that reported in other studies. It was concluded that the arsenic entered the subjects as dust which is prevalent in the Bombay area.

Metal levels are also found to correlate with disease states. Nakahara et al. (191) found the metals Rb, Mn, Fe, Co, Cu, Zn, Al and Se to be low in the blood serum of cancer patients relative to controls. Cr, Mn, Fe, Co, Zn, Cu and Sb were similarly found to be low in subjects with Down's syndrome.

Cadmium

Evaluations were performed to determine which biological matrix was the best indicator of cadmium exposure (83,84). Although amounts of cadmium excreted in the urine per day are low (1-2 μg) and not particularly sensitive to acute exposure, they can suggest the absence or presence of cadmium-induced renal dysfunction (84-86). Cadmium in urine was correlated with actual body burden while blood cadmium was shown to be a good indicator of average recent intake over a three-month period (87).

Residents living near a cadmium smelter facility did not have significantly higher cadmium levels in blood or urine than a control group. Any increase was apparently dwarfed by the effect of cigarette smoking (which increases blood cadmium fivefold) (88). However, cadmium levels in blood and hair were significantly higher in children around zinc smelters (89). In another study, cadmium levels in blood and urine of children living in smelter communities gave a direct correlation with cadmium content in dirt and residues on fingers (76). Urinary cadmium content in elderly subjects was higher from areas contaminated with cadmium (nonferrous smelters) compared to control areas (90).

Significant correlations exist between urinary cadmium levels and certain occupational exposures due to airborne metals. Cadmium content in hair and blood did not correlate as well with exposure (91). Cadmium levels in urine and blood are significantly higher in occupationally exposed groups (92). Blood and urine cadmium content were higher in employees working in factories producing cadmium salts (93,94). Cadmium in blood was related to occupational exposure in a jewelry factory (95) although plasma levels were not higher (95). Cadmium in blood samples from cadmium workers was correlated with exposure (97). Urinary cadmium in occupationally-exposed workers decreased remarkably after process improvement resulted in a 90% reduction of airborne cadmium (98).

No association was found between cadmium in hair and in dustfall in the New York City area (99). Average urinary cadmium was higher in Japanese villagers who consumed high cadmium-containing rice (100).

Calcium and Magnesium

Calcium and magnesium levels in scalp hair have been studied as a possible index to body burden and exposure (101).

Chromium

Urinary chromium may be useful in detecting very recent exposure (103). A close relationship was shown to exist between chromium levels in urine and airborne chromium (103). A correlation was established between chromium in hair from children and dustfall in the New York City area (100).

Skin autopsy samples from a chromate worker had significantly elevated chromium levels compared to a control (105). Blood chromium was significantly higher in autoworkers than in a control group (106).

Determination of Cr(VI) and Cr(III) in human urine and in commercial dextrose solution was performed after selective preconcentration of the chromium species at different pH values by poly(dithiocarbamate) and poly(acrylamidoxime) chelating agents (107). Chromium in human urine was found to exist exclusively as Cr(III).

Lead

Exposure of Children--

Children, particularly preschoolers, are susceptible to metal exposure (102). Overall body concentration of a metal pollutant will tend to be higher than for adults since the same environmental input is distributed over a smaller frame. In addition, small children will make contact with the dirt and surfaces contaminated by air particulates and will very often place a metal-contaminated hand in the mouth.

Epidemiological studies often show correlations of environmental metal levels with levels in the biological fluids from children but not adults. Since children represent a special at risk group, this subsection will contain literature citations specifically assessing exposure to this age group.

Lead levels in blood from children living in smelter communities were correlated with air, dustfall, soil, and drinking water (108-110) although the association was significant only with dustfall and soil in urban setting (111). Blood lead values were also found to be correlated with lead concentration in drinking water (112). Lead concentrations in hair were related to lead levels in dustfall (99). The concentration of lead in blood and hair were significantly higher in residents living near a zinc smelter compared to a control area (77). The blood lead measurements in children from another smelter community indicated undue exposure (113). Preschoolers living near a lead works or in the same house as a lead worker were shown to have elevated lead levels in blood (114). Studies have shown that children whose parent(s) worked at a storage battery factory had high blood lead levels (111). Children with a history of pica showed elevated lead levels in blood (116).

Children living in poor housing with lead paint showed high lead levels in blood (117,118). Elevated blood levels were also found in children living in houses where lead-painted wood was burned as a fuel (119).

Studies have established a relationship between inhalation of automobile exhaust and lead levels in children's hair (120,121).

Occupational Exposure--

Lead levels in blood are the most common indicator of occupational exposure. A listing of biological matrices used to monitor exposure in the workplace is given in Table 17-1.

General--

Blood and hair have been the most commonly used biological indicators for identifying lead exposure.

Blood--Lead in blood remains the single most readily available, widely used, and easily interpreted index of lead burden (149), particular for short term exposure (150).

Lead in blood levels have been used to assess the lead status in an exposed and control population (151) and in residents living near a nonferrous metal plant (152).

Blood lead values have been used to evaluate airborne lead exposure (153) and to estimate automotive sources of lead emission (154). The significant difference between urban and rural blood lead concentrations is due to lead exposure from dust and dirt (155).

Potential contamination is possible when collecting capillary blood for testing (156).

Hair--Scalp hair has been investigated as a means for estimating overall lead exposure and segmental analysis for determining time of exposure (157). Hair samples provide a good indicator of exposure to lead over long periods of time (158).

A correlation was established between lead levels in hair and certain population groups (159). Increasing hair lead values were thought to be due to motor traffic (160).

Other matrices--Significant correlations of airborne concentrations of lead were found with lead values in urine, hair, and blood in different population groups (91). Studies have been carried out to determine the usefulness of plasma as a measure of lead exposure (161). Lead in teeth may provide a means of estimating long-term exposure (150).

Mercury

A study of mercury in the urine of caustic soda plant workers was performed by Lo et al. (187). They found that the content was highest among those workers who performed electrolysis cell reconditioning. They found, too, that the levels increased with the size of the electrolysis cells being cleaned. It was determined that the mercury was transported to the workers principally through the air.

Hair samples taken from citizens of Chile were analyzed for arsenic and mercury levels by Cortes et al. (188). They found the levels of these metals low in samples taken from subjects in the vacinity of Santiago, which is relatively pollution free. Hair from individuals exposed to heavy metal pollution were found to have elevated levels of these two metals; it was thought that water was the principal source of these metals.

Nickel

Both urine and serum were evaluated as indicators of nickel ingestion. Urine was shown to be the best matrix for identifying and following the course of a single acute dose of a soluble nickel salt (162). Nickel concentration in urine was evaluated as a means for monitoring exposure to nickel (165). Both nickel in urine and plasma can be used as biological indicators of exposure (168,169). A correlation was established between nickel content in hair from children and dustfall in the New York City area (99). Blood nickel in autoworkers were significantly higher than controls (106).

Palladium

An epidemiological study was conducted to determine palladium levels in blood, urine, and hair. All analytical measurements were below the detection limit of the method (164).

Silver

Silver concentration in blood increased with length of employment in occupations where exposure to silver nitrate and silver oxide were likely (163). No association was found between silver levels in hair and dustfall in the New York City area (99).

Vanadium

A correlation was established between vanadium content in children and adult hair and dustfall in the New York City area (99).

Cornelis et al. (189) studied the vanadium levels in the blood serum of groups of men and women. The range found for the women was 0.017 to 0.055 ng/mL, whereas the range for the men was found to be 0.029 to 0.939 ng/mL. This elevated level in the men was apparently due to the fact that a number of them used vanadium-containing steel tools.

Zinc

The significantly higher levels of zinc in the hair of Bushman women compared to inner city US women may be due to the use of iron pots for cooking in Botswana (162). Hair has been evaluated as an indicator of zinc exposure (167) and has been used to monitor environmental contamination (158). A study has been carried out to identify problems in interpreting zinc hair data (101). Zinc levels in blood and urine were monitored after feeding zinc sulfate to experimental subjects (166).

No association was observed between zinc in hair and dustfall in the New York City area (99). No correlation was found between zinc levels in hair, blood, or urine and exposure (91).

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